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(54) Title: CAULIFLOWER FLORAL MERISTEM IDENTITY GENES AND METHODS OF USING SAME

(57) Abstract

The present invention provides a nucleic acid molecule encoding a CAULIFLOWER (CAL) gene product such as a nucleic acid molecule encoding *Arabidopsis thaliana* CAL and a nucleic acid molecule encoding *Brassica oleracea* CAL (BoCAL). The invention also provides a nucleic acid molecule encoding a truncated CAL gene product such as a nucleic acid molecule encoding *Brassica oleracea* var. *botrytis* CAL (BobCAL). The invention also provides a nucleic acid containing the *Arabidopsis thaliana CAL* gene, a nucleic acid molecule containing the *Brassica oleracea CAL* gene and a nucleic acid molecule containing the *Brassica oleracea var. botritis CAL* gene. The invention further provides a kit for converting shoot meristem to floral meristem and a kit for promoting early flowering in an angiosperm. The invention provides a CAL polypeptide and an antibody that specifically binds CAL polypeptides. In addition, the invention provides the truncated BobCAL polypeptide and an antibody that specifically binds truncated BobCAL polypeptide. The invention further provides a method of identifying a *Brassica* having a modified *CAL* CAL allele by detecting a polymorphism associated with a *CAL* CAL locus, where the *CAL* CAL locus comprises a modified *CAL* CAL allele that does not encode an active CAL gene product.

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CAULIFLOWER FLORAL MERISTEM IDENTITY GENES AND METHODS OF USING SAME

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BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates generally to the field of plant flowering and more specifically to genes involved in the regulation of flowering.

BACKGROUND INFORMATION

A flower is the reproductive structure of a flowering plant. Following fertilization, the ovary of the flower becomes a fruit and bears seeds. As a practical consequence, production of fruit and seed-derived crops such as grapes, beans, corn, wheat and rice is dependent upon flowering.

Early in the plant life cycle, vegetative growth occurs, and roots, stems and leaves are formed. During the later period of reproductive growth, flowers as well as new shoots or branches develop. However, the factors responsible for the transition from vegetative to reproductive growth, and the onset of flowering, are poorly understood.

A variety of external signals, such as length of daylight and temperature, affect the time of flowering. The time of flowering also is subject to genetic controls that prevent young plants from flowering prematurely. Thus, the pattern of genes expressed in a plant is an important determinant of the time of flowering.

Given these external signals and genetic controls, a relatively fixed period of vegetative growth precedes flowering in a particular plant species. The length of time required for a crop to mature to flowering limits the geographic location in which it can be grown and can be an important determinant of yield. In addition, since the time of flowering determines when a plant is reproductively mature, the pace of a plant breeding program also depends upon the length of time required for a plant to flower.

Traditionally, plant breeding involves generating hybrids of existing plants, which are examined for improved yield or quality. The improvement of existing plant crops through plant breeding is central to increasing the amount of food grown in the world since the amount of land suitable for agriculture is limited. For example, the development of new strains of wheat, corn and rice through plant breeding has increased the yield of these crops grown in underdeveloped countries such as Mexico, India and Pakistan. Unfortunately, plant breeding is inherently a slow process since plants must

be reproductively mature before selective breeding can proceed.

For some plant species, the length of time needed to mature to flowering is so long that selective breeding, which requires several rounds of backcrossing progeny plants with their parents, is impractical. For example, perennial trees such as walnut, hickory, oak, maple and cherry do not flower for several years after planting. As a result, breeding of such plant species for insect or disease-resistance or to produce improved wood or fruit, for example, would require many years, even if only a few rounds of selection were performed.

Methods of promoting early flowering can make breeding of long generation plants such as trees practical for the first time. Methods of promoting early flowering also would be useful for shortening growth periods, thereby broadening the geographic range in which a crop such as rice, corn or coffee can be grown. Unfortunately, methods for promoting early flowering in a plant have not yet been described. Thus, there is a need for methods that promote early flowering. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a nucleic acid molecule encoding a CAULIFLOWER (CAL) gene product. For example, the invention provides a nucleic acid molecule encoding *Arabidopsis thaliana* CAL and a nucleic acid molecule encoding *Brassica oleracea* CAL.

The invention also provides a nucleic acid molecule encoding a truncated CAL gene product. For example, the invention provides a nucleic acid molecule encoding the truncated *Brassica oleracea var. botrytis* CAL gene product. The invention also provides a nucleotide sequence that hybridizes under relatively stringent conditions to a nucleic acid molecule encoding a CAL gene product, a truncated CAL gene product, or a complementary sequence thereto.

The invention further provides the *Arabidopsis thaliana CAL* gene, *Brassica oleracea CAL* gene and *Brassica oleracea var. botrytis CAL* gene. In addition, the invention provides a nucleotide sequence that hybridizes under relatively stringent conditions to the *Arabidopsis thaliana CAL* gene, *Brassica oleracea CAL* gene or *Brassica oleracea var. botrytis CAL* gene, or a complementary sequence thereto.

The invention also provides vectors, including expression vectors, containing a nucleic acid molecule encoding a CAL gene product. The invention further provides a kit for converting shoot meristem to floral meristem in an angiosperm and a kit for promoting early flowering in an angiosperm.

In addition, the invention provides a CAL polypeptide, such as the *Arabidopsis thaliana* CAL polypeptide or the *Brassica oleracea* CAL polypeptide, as well as an antibody that specifically binds a CAL polypeptide. The invention further provides the truncated *Brassica oleracea var. botrytis* CAL polypeptide and an antibody that specifically binds the truncated *Brassica oleracea var. botrytis* CAL polypeptide.

The invention further provides a method of identifying a *Brassica* having a modified *CAL* allele by detecting a polymorphism associated with a *CAL* locus, where the *CAL* locus comprises a modified *CAL* allele that does not encode an active CAL gene product. For example, the polymorphism can be a restriction fragment length polymorphism and the modified *CAL* allele can be the *Brassica oleracea var. botrytis CAL* allele.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the nucleotide (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2) sequence of the *Arabidopsis thaliana AP1* cDNA.

Figure 2 illustrates the nucleotide (SEQ ID NO: 3) and amino acid (SEQ ID NO: 4) sequence of the *Brassica oleracea AP1* cDNA.

Figure 3 illustrates the nucleotide (SEQ ID NO: 5) and amino acid (SEQ ID NO: 6) sequence of the *Brassica oleracea* var. *botrytis AP1* cDNA.

Figure 4 illustrates the nucleotide (SEQ ID NO: 7) and amino acid (SEQ ID NO: 8) sequence of the *Zea mays AP1* cDNA. The GenBank accession number is L46400.

Figure 5 illustrates the nucleotide (SEQ ID NO: 9) and amino acid (SEQ ID NO: 10) sequence of the *Arabidopsis thaliana CAL* cDNA.

Figure 6 illustrates the nucleotide (SEQ ID NO: 11) and amino acid (SEQ ID NO: 12) sequence of the *Brassica oleracea CAL* cDNA.

Figure 7 illustrates the nucleotide (SEQ ID NO: 13) and amino acid (SEQ ID NO: 14) sequence of the *Brassica oleracea* var. *botrytis CAL* cDNA.

Figure 8 illustrates *CAL* gene structure and provides a comparison of various CAL amino acid sequences.

Figure 8A. Exon-intron structure of *Arabidopsis CAL* gene. Exons are shown as boxes and introns as a solid line. Sizes (in base pairs) are

indicated above. Locations of changes resulting in mutant alleles are indicated by arrows. MADS and K domains are hatched.

Figure 8B. An alignment of three deduced amino acid sequences of *CAL* cDNAs. The complete *Arabidopsis thaliana CAL* amino acid sequence is displayed. The *Brassica oleracea CAL (BoCAL)* and *Brassica oleracea* var. *botrytis CAL (BobCAL)* amino acid sequences are shown directly below the *Arabidopsis* sequence where the sequences differ. The *AP1* amino acid sequence is shown for comparison. The MADS domain is indicated in bold and the K domain is underlined. GenBank accession numbers are as follows: *Arabidopsis thaliana CAL* (L36925); *Brassica oleracea CAL* (L36926) and *Brassica oleracea* var. *botrytis CAL* (L36927).

Figure 9 illustrates the nucleotide (SEQ ID NO: 15) and amino acid (SEQ ID NO: 16) sequence of the *Arabidopsis thaliana LEAFY (LFY)* cDNA.

Figure 10 illustrates the genomic sequence of *Arabidopsis thaliana AP1* (SEQ ID NO: 17).

Figure 11 illustrates the genomic sequence of *Brassica oleracea AP1* (SEQ ID NO: 18).

Figure 12 illustrates the genomic sequence of *Brassica oleracea* var. *botrytis AP1* (SEQ ID NO: 19).

Figure 13 illustrates the genomic sequence of *Arabidopsis thaliana CAL* (SEQ ID NO: 20).

Figure 14 illustrates the genomic sequence of *Brassica oleracea CAL* (SEQ ID NO: 21).

Figure 15 illustrates the genomic sequence of *Brassica oleracea* var. *botrytis CAL* (SEQ ID NO: 22).

Figure 16 illustrates the nucleotide (SEQ ID NO: 23) and amino acid (SEQ ID NO: 24) sequence of the rat glucocorticoid receptor ligand binding domain.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a nucleic acid molecule encoding a CAULIFLOWER (CAL) gene product, which is a floral meristem identity gene product involved in the conversion of shoot meristem to floral meristem. For example, the invention provides a nucleic acid molecule encoding *Arabidopsis thaliana* CAL and a nucleic acid molecule encoding *Brassica oleracea* CAL (BoCAL) (Kempin et al., Science, 267:522-525 (1995), which is incorporated herein by reference). As disclosed herein, a CAL gene product can be expressed in an angiosperm, thereby converting shoot meristem to floral meristem in the angiosperm or promoting early flowering in the angiosperm. The invention also provides a nucleic acid molecule encoding a truncated CAL gene product such as a nucleic acid molecule encoding *Brassica oleracea* var. *botrytis* CAL (BobCAL). The invention also provides a

nucleic acid molecule containing the *Arabidopsis thaliana* CAL gene, a nucleic acid molecule containing the *Brassica oleracea CAL* gene and a nucleic acid molecule containing the *Brassica oleracea var. botrytis CAL* gene. The invention further provides a kit for converting shoot meristem to floral meristem and a kit for promoting early flowering in an angiosperm. The invention provides a CAL polypeptide and an antibody that specifically binds CAL polypeptide. In addition, the invention provides the truncated BobCAL polypeptide and an antibody that specifically binds the truncated BobCAL polypeptide. The invention further provides a method of identifying a *Brassica* having a modified *CAL* allele by detecting a polymorphism associated with a *CAL* locus, where the *CAL* locus comprises a modified *CAL* allele that does not encode an active CAL gene product.

The present invention provides a non-naturally occurring angiosperm containing a first ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product. For example, the invention provides a transgenic angiosperm containing a first ectopically expressible floral meristem identity gene product such as APETALA1 (AP1), CAULIFLOWER (CAL) or LEAFY (LFY). Such a transgenic angiosperm can be, for example, a cereal plant, leguminous plant, oilseed plant, tree, fruit-bearing plant or ornamental flower.

A flower, like a leaf or shoot, is derived from the shoot apical meristem, which is a collection of undifferentiated cells set aside during embryogenesis.

The production of v getative structures, such as leaves or shoots, and of reproductive structures, such as flowers, is temporally segregated, such that a leaf or shoot arises early in a plant life cycle, while a flower develops later. The transition from vegetative to reproductive development is the consequence of a process termed floral induction (Yanofsky, Ann. Rev. Plant Physiol. Plant Mol. Biol. 46:167-188 (1995)).

Once induced, shoot apical meristem either persists and produces floral meristem, which gives rise to flowers, and lateral meristem, which gives rise to branches, or is itself converted to floral meristem. The fate of floral meristem is to differentiate into a single flower having a fixed number of floral organs in a whorled arrangement. Dicots, for example, contain four whorls (concentric rings) in which sepals (first whorl) and petals (second whorl) surround stamens (third whorl) and carpels (fourth whorl).

Although shoot meristem and floral meristem both consist of meristemic tissue, shoot meristem is distinguishable from the more specialized floral meristem. Shoot meristem generally is indeterminate and gives rise to an unspecified number of floral and lateral meristems. In contrast, floral meristem is determinate and gives rise to the fixed number of floral organs that comprise a flower.

By convention herein, a wild-type gene sequence is represented in upper case italic letters (for example,

APETALA1), and a wild-type gene product is represented in upper case non-italic letters (APETALA1). Further, a mutant gene allele is represented in lower case italic letters (*ap1*), and a mutant gene product is represented in lower case non-italic letters (ap1).

Genetic studies have identified a number of genes involved in regulating flower development. These genes can be classified into different groups depending on their function. Flowering time genes, for example, are involved in floral induction and regulate the transition from vegetative to reproductive growth. In comparison, the floral meristem identity genes, which are the subject matter of the present invention as disclosed herein, encode proteins that promote the conversion of shoot meristem to floral meristem. In addition, floral organ identity genes encode proteins that determine whether sepals, petals, stamens or carpels are formed (Yanofsky, *supra*, 1995; Weigel, Ann. Rev. Genetics 29:19-39 (1995)). Some of the floral meristem identity gene products also have a role in specifying organ identity.

Floral meristem identity genes have been identified by characterizing genetic mutations that prevent or alter floral meristem formation. Among floral meristem identity gene mutations in *Arabidopsis thaliana*, those in the gene *LEAFY (LFY)* generally have the strongest effect on floral meristem identity. Mutations in *LFY* completely transform the basal-most flowers into secondary shoots and have variable effects on

later-arising (apical) flowers. In comparison, mutations in the floral meristem identity gene *APETALA1 (AP1)* result in replacement of a few basal flowers by inflorescence shoots that are not subtended by leaves. An apical flower produced in an *ap1* mutant has an indeterminate structure in which a flower arises within a flower. These mutant phenotypes indicate that both *AP1* and *LFY* contribute to establishing the identity of the floral meristem although neither gene is absolutely required. The phenotype of *lfy ap1* double mutants, in which structures with flower-like characteristics are very rare, indicates that *LFY* and *AP1* encode partially redundant activities.

In addition to the *LFY* and *AP1* genes, a third locus that greatly enhances the *ap1* mutant phenotype has been identified in *Arabidopsis*. This locus, designated *CAULIFLOWER (CAL)*, derives its name from the resulting "cauliflower" phenotype, which is strikingly similar to the common garden variety of cauliflower. In an *ap1 cal* double mutant, floral meristem that develops behaves as shoot meristem in that there is a massive proliferation of meristems in the position that normally would be occupied by a single flower. However, a plant homozygous for a particular *cal* mutation (*cal-1*) has a normal phenotype, indicating that AP1 can substitute for the loss of CAL in these plants. In addition, because floral meristem that forms in an *ap1* mutant behaves as shoot meristem in an *ap1 cal* double mutant, CAL can largely substitute for AP1 in specifying floral meristem. These genetic data indicate that *CAL* and *AP1* encode activities

that are partially redundant in converting shoot meristem to floral meristem.

Other genetic loci play at least minor roles in specifying floral meristem identity. For example, although a mutation in *APETALA2 (AP2)* alone does not result in altered inflorescence characteristics, *ap2 ap1* double mutants have indeterminate flowers (flowers with shoot-like characteristics) (Bowman et al., Development 119:721-743 (1993)). Also, mutations in the *CLAVATA1* (*CLV1*) gene result in an enlarged meristem and lead to a variety of phenotypes (Clark et al., Development 119:397-418 (1993)). In a *clv1 ap1* double mutant, formation of flowers is initiated, but the center of each flower often develops as an indeterminate inflorescence. Thus, mutations in *CLAVATA1* result in the loss of floral meristem identity in the center of wild-type flowers. Genetic evidence also indicates that the gene product of *UNUSUAL FLORAL ORGANS (UFO)* plays a role in determining the identity of floral meristem. Additional floral meristem identity genes associated with altered floral meristem formation remain to be isolated.

Mutations in another locus, designated *TERMINAL FLOWER (TFL)*, produce phenotypes that generally are reversed as compared to mutations in the floral meristem identity genes. For example, *tfl* mutants flower early, and the indeterminate apical and lateral meristems develop as determinate floral meristems (Alvarez et al., Plant J. 2:103-116 (1992)). These characteristics indicate that the TFL promotes maintenance of shoot

meristem. *TFL* also acts dir ctly or indirectly to negatively regulate AP1 and LFY expression in shoot meristem since AP1 and LFY are ectopically expressed in the shoot meristem of *tfl* mutants (Gustafson-Brown et al., Cell 76:131-143 (1994); Weigel et al., Cell 69:843-859 (1992)). It is recognized that a plant having a mutation in TFL can have a phenotype similar to a non-naturally occurring angiosperm of the invention. Such *tfl* mutants, however, are explicitly excluded from the scope of the present invention.

The results of such genetic studies indicate that several floral meristem identity gene products, including AP1, CAL and LFY, act redundantly to convert shoot meristem to floral meristem and that TFL acts directly or indirectly to negatively regulate expression of the floral meristem identity genes. As disclosed herein, ectopic expression of a single floral meristem identity gene product such as AP1, CAL or LFY is sufficient to convert shoot meristem to floral meristem. Thus, the present invention provides a non-naturally occurring angiosperm that contains an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product, provided that such ectopic expression is not due to a mutation in an endogenous *TERMINAL FLOWER* gene.

As disclosed herein, an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product can be, for example, a transgene encoding a floral meristem identity gene product under control of a

heterologous gene regulatory element. In addition, such an ectopically expressible nucleic acid molecule can be an endogenous floral meristem identity gene coding sequence that is placed under control of a heterologous gene regulatory element. The ectopically expressible nucleic acid molecule also can be, for example, an endogenous floral meristem identity gene having a modified gene regulatory element such that the endogenous floral meristem identity gene is no longer subject to negative regulation by TFL.

The term "ectopically expressible" is used herein to refer to a gene transcript or gene product that can be expressed in a tissue other than a tissue in which it normally is produced. The actual ectopic expression thereof is dependent on various factors and can be constitutive or inducible expression. As disclosed herein, AP1, which normally is expressed in floral meristem, is ectopically expressible in shoot meristem. As disclosed herein, when a floral meristem identity gene product such as AP1, CAL or LFY is ectopically expressed in shoot meristem, the shoot meristem is converted to floral meristem and early flowering can occur (see Examples II, IV and V).

In particular, an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product can be expressed prior to the developmental time at which the corresponding endogenous gene normally is expressed. For example, an *Arabidopsis* plant grown under continuous light conditions expresses *AP1* just

prior to day 18, when normal flowering begins. However, as disclosed herein, *AP1* can be ectopically expressed in shoot meristem earlier than day 18, resulting in early conversion of shoot meristem to floral meristem and early flowering. As shown in Example IID, a transgenic *Arabidopsis* plant that ectopically expresses *AP1* in shoot meristem under control of a constitutive promoter flowers earlier than the corresponding non-transgenic plant (day 10 as compared to day 18).

As used herein, the term "floral meristem identity gene product" means a gene product that promotes conversion of shoot meristem to floral meristem. As disclosed herein, expression of a floral meristem identity gene product such as AP1, CAL or LFY in shoot meristem can convert shoot meristem to floral meristem. Furthermore, expression of a floral meristem identity gene product in shoot meristem also can promote early flowering (Examples IID, IVA and V). A floral meristem identity gene product is distinguishable from a late flowering gene product or an early flowering gene product, which are not encompassed within the present invention. In addition, reference is made herein to an "inactive" floral meristem identity gene product, as exemplified by BobCAL (see below). Expression of an inactive floral meristem identity gene product in an angiosperm does not result in the conversion of shoot meristem to floral meristem in the angiosperm.

A floral meristem identity gene product can be, for example, an AP1 gene product such as *Arabidopsis* AP1,

which is a 256 amino acid gene product encoded by the *AP1* cDNA sequence isolated from *Arabidopsis thaliana* (Figure 5, SEQ ID NO: 2). The *Arabidopsis AP1* cDNA encodes a highly conserved MADS domain, which can function as a DNA-binding domain, and a K domain, which is structurally similar to the coiled-coil domain of keratins and can be involved in protein-protein interactions.

In *Arabidopsis*, *AP1* RNA is expressed in flowers but is not detectable in roots, stems or leaves (Mandel et al., Nature 360:273-277 (1992), which is incorporated herein by reference). The earliest detectable expression of *AP1* RNA is in young floral meristem at the time it initially forms on the flanks of shoot meristem. Expression of *AP1* increases as the floral meristem increases in size; no *AP1* expression is detectable in shoot meristem. In later stages of development, *AP1* expression ceases in cells that will give rise to reproductive organs (stamens and carpels), but is maintained in cells that will give rise to non-reproductive organs (sepals and petals; Mandel, *supra*, 1992).

As used herein, the term "APETALA1" or "AP1" means a floral meristem identity gene product that is characterized, in part, by having an amino acid sequence that is related to the *Arabidopsis* AP1 amino acid sequence shown in Figure 1 (SEQ ID NO: 2) or to the *Zea mays* AP1 amino acid sequence shown in Figure 4 (SEQ ID NO: 8). In nature, AP1 is expressed in floral meristem.

CAULIFLOWER (CAL) is another example of a floral meristem identity gene product. As used herein, the term "CAULIFLOWER" or "CAL" means a floral meristem identity gene product that is characterized in part by having an amino acid sequence that has at least about 70 percent identity with the amino acid sequence shown in Figure 5 (SEQ ID NO: 10) in the region from amino acid 1 to amino acid 160 or with the amino acid sequence shown in Figure 6 (SEQ ID NO: 12) in the region from amino acid 1 to amino acid 160. In nature, CAL is expressed in floral meristem.

The present invention provides a nucleic acid molecule encoding a CAL, including, for example, the *Arabidopsis CAL* cDNA sequence shown in Figure 5 (SEQ ID NO: 9). As disclosed herein, CAL, like AP1, contains a MADS domain and a K domain. The MADS domains of CAL and AP1 differ in only five of 56 amino acid residues, where four of the five differences represent conservative amino acid replacements. Over the entire sequence, the *Arabidopsis* CAL and *Arabidopsis* AP1 sequences (SEQ ID NOS: 10 and 2) are 76% identical and are 88% similar if conservative amino acid substitutions are allowed.

Similar to the expression pattern of *AP1*, *CAL* RNA is expressed in young floral meristem in *Arabidopsis*. However, in contrast to *AP1* expression, which is high throughout sepal and petal development, *CAL* expression is low in these organs.

LEAFY (LFY) is yet another example of a floral meristem identity gene product. As used herein, the term "LEAFY" or "LFY" means a floral meristem identity gene product that is characterized in part by having an amino acid sequence that is related to the amino acid sequence shown in Figure 9 (SEQ ID NO: 16) In nature, LFY is expressed in floral meristem as well as during vegetative development. As disclosed herein, ectopic expression of floral meristem identity gene products, which normally are expressed in floral meristem, such as AP1 or CAL or LFY or combinations thereof, in shoot meristem can convert shoot meristem to floral meristem and promote early flowering.

Flower development in *Arabidopsis* is recognized in the art as a model for flower development in angiosperms in general. Gene orthologs corresponding to the *Arabidopsis* genes involved in the early steps of flower formation have been identified in distantly related plant species, and these gene orthologs show remarkably similar RNA expression patterns. Mutations in these genes also result in phenotypes that correspond to the phenotype produced by a similar mutation in *Arabidopsis*. For example, orthologs of the *Arabidopsis* floral meristem identity genes *AP1* and *LFY* and the *Arabidopsis* organ identity genes *AGAMOUS*, *APETALA3* and PISTILLATA have been isolated from monocots such as maize and, where characterized, reveal the anticipated RNA expression patterns and related mutant phenotypes. (Schmidt et al., Plant Cell 5:729-737 (1993); and Veit et al., Plant Cell 5:1205-1215 (1993), each of which is

incorporated herein by reference). Furthermore, a gene ortholog can be functionally interchangeable in that it can function across distantly related species boundaries (Mandel et al., Cell 71:133-143 (1992), which is incorporated herein by reference). Taken together, these data suggest that the underlying mechanisms controlling the initiation and proper development of flowers are conserved across distantly related dicot and monocot boundaries. Therefore, results obtained using *Arabidopsis* can be predictive of results that can be expected in other angiosperms.

Floral meristem identity genes in particular are conserved throughout the plant kingdom. For example, a gene ortholog of *Arabidopsis AP1* has been isolated from *Antirrhinum majus* (snapdragon; Huijser et al., EMBO J. 11:1239-1249 (1992), which is herein incorporated by reference). As disclosed herein, an ortholog of *Arabidopsis AP1* also has been isolated from *Zea Mays* (maize; see Example IA). Similarly, gene orthologs of *Arabidopsis LFY* have been isolated from *Antirrhinum majus*, tobacco and poplar tree (Coen et al., Cell, 63:1311-1322 (1990); Kelly et al., Plant Cell 7:225-234 (1995); and Strauss et al., Molec. Breed 1:5-26 (1995), each of which is incorporated herein by reference). In addition, a mutation in the *Antirrhinum AP1* ortholog results in a phenotype similar to the *Arabidopsis ap1* mutant phenotype described above (Huijser et al., *supra*, 1992). Similarly, a mutation in the *Antirrhinum LFY* ortholog results in a phenotype similar to the *Arabidopsis lfy* mutant phenotype (Coen et al., *supra*,

1995). These studies indicate that AP1 and LFY function similarly in distantly related angiosperms.

A floral meristem identity gene product also can function across species boundaries. For example, *Arabidopsis* LFY can convert shoot meristem to floral meristem when expressed in aspen trees (Weigel and Nilsson, Nature 377:495-500 (1995), which is incorporated herein by reference). As disclosed herein, a nucleic acid molecule encoding an *Arabidopsis* AP1 or CAL gene product (SEQ ID NOS: 1 and 9), for example, also can be used to convert shoot meristem to floral meristem in an angiosperm. Thus, a nucleic acid molecule encoding an *Arabidopsis* AP1 gene product (SEQ ID NO: 1) or an *Arabidopsis* CAL gene product (SEQ ID NO: 9) can be introduced into an angiosperm such as corn, wheat or rice and, upon expression, can convert shoot meristem to floral meristem in the transgenic angiosperm. Furthermore, as disclosed herein, the conserved nature of an *AP1* or *CAL* or *LFY* gene among diverse angiosperms, allows a nucleic acid molecule encoding a floral meristem identity gene product from essentially any angiosperm to be introduced into essentially any other angiosperm, wherein the expression of the nucleic acid molecule in shoot meristem can convert shoot meristem to floral meristem.

If desired, a novel *AP1*, *CAL* or *LFY* sequence can be isolated from an angiosperm using a nucleotide sequence as a probe and methods well known in the art of molecular biology (Sambrook et al. (eds.), Molecular

Cloning: A Laboratory Manual (Second Edition), Plainview, NY: Cold Spring Harbor Laboratory Press (1989), which is herein incorporated by reference). As exemplified herein and discussed in detail below (see Example IA), the *AP1* ortholog from *Zea Mays* (maize; SEQ ID NO: 7) was isolated using the *Arabidopsis AP1* cDNA as a probe (SEQ ID NO: 1).

In one embodiment, the invention provides a non-naturally occurring angiosperm that contains an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product and that is characterized by early flowering. As used herein, the term "characterized by early flowering," when used in reference to a non-naturally occurring angiosperm of the invention, means a non-naturally occurring angiosperm that forms flowers sooner than flowers would form on a corresponding naturally occurring angiosperm that does not ectopically express a floral meristem identity gene product, grown under the same conditions. Flowering times for naturally occurring angiosperms are well known in the art and depend, in part, on genetic factors and on the environmental conditions, such as day length. Thus, given a defined set of environmental conditions, a naturally occurring plant will flower at a relatively predictable time.

It is recognized that various transgenic plants that are characterized by early flowering have been described. Such transgenic plants are described herein and are readily distinguishable or explicitly excluded

from the present invention. For example, a product of a "late-flowering gene" can promote early flowering but does not specify the conversion of shoot meristem to floral meristem. Therefore, a transgenic plant expressing a late-flowering gene product is distinguishable from a non-naturally occurring angiosperm of the invention. For example, a transgenic plant expressing the late-flowering gene, *CONSTANS (CO)*, flowers earlier than a corresponding wild type plant (Putterill et al., Cell 80:847-857 (1995)). However, expression of exogenous *CONSTANS* does not convert shoot meristem to floral meristem.

Early flowering also has been observed in a transgenic tobacco plant expressing an exogenous rice MADS domain gene. Although the product of this gene promotes early flowering, it does not specify the identity of floral meristem and, thus, cannot convert shoot meristem to floral meristem (Chung et al., Plant Mol. Biol. 26:657-665 (1994)). Therefore, the early-flowering CO and rice MADS domain gene transgenic plants are distinguishable from the early-flowering non-naturally occurring angiosperms of the invention.

Mutations in a class of genes known as "early-flowering genes" also result in plants that flower prematurely. Such early flowering genes include, for example, *EARLY FLOWERING 1-3 (ELF1, ELF2, ELF3); EMBRYONIC FLOWER 1,2 (EMF1, EMF2); LONG HYPOCOTYL 1,2 (HY1, HY2); PHYTOCHROME B (PHYB), SPINDLY (SPY)* and *TERMINAL FLOWER (TFL)* (Weigel, *supra*, 1995). However,

the wild type product of an early flowering gene retards flowering and is distinguishable from a floral meristem identity gene product in that it does not promote conversion of shoot meristem to floral meristem.

An *Arabidopsis* plant having a mutation in the *TERMINAL FLOWER (TFL)* gene flowers early and is characterized by the conversion of shoots to flowers (Alvarez et al., Plant J. 2:103-116 (1992), which is incorporated herein by reference). However, TFL is not a floral meristem identity gene product, as defined herein. Specifically, it is the loss of TFL that promotes conversion of shoot meristem to floral meristem. Since the function of TFL is to antagonize formation of floral meristem, a *tfl* mutant, which has lost this antagonist function, permits conversion of shoot meristem to floral meristem. Although TFL is not a floral meristem identity gene product and does not itself convert shoot meristem to floral meristem, the loss of TFL can result in a plant with an ectopically expressed floral meristem identity gene product. Such *tfl* mutants, in which a mutation in *TFL* results in conversion of shoot meristem to floral meristem, are explicitly excluded from the present invention.

As used herein, the term "non-naturally occurring angiosperm" means an angiosperm that contains a genome that has been modified by man. A transgenic angiosperm, for example, contains an exogenous nucleic acid molecule and, therefore, contains a genome that has been modified by man. Furthermore, an angiosperm that

contains, for example, a mutation in an endogenous floral meristem identity gene regulatory element as a result of exposure to a mutagenic agent by man also contains a genome that has been modified by man. In contrast, a plant containing a spontaneous or naturally occurring mutation is not a "non-naturally occurring angiosperm" and, therefore, is not encompassed within the invention.

As used herein, the term "transgenic" refers to an angiosperm that contains in its genome an exogenous nucleic acid molecule, which can be derived from the same or a different species. The exogenous nucleic acid molecule that is introduced into the angiosperm can be a gene regulatory element such as a promoter or other regulatory element or can be a coding sequence, which can be linked to a heterologous gene regulatory element.

As used herein, the term "angiosperm" means a flowering plant. Angiosperms are well known and produce a variety of useful products including materials such as lumber, rubber, and paper; fibers such as cotton and linen; herbs and medicines such as quinine and vinblastine; ornamental flowers such as roses and orchids; and foodstuffs such as grains, oils, fruits and vegetables.

Angiosperms are divided into two broad classes based on the number of cotyledons, which are seed leaves that generally store or absorb food. Thus, a monocotyledonous angiosperm is an angiosperm having a

single cotyledon, and a dicotyledonous angiosperm is an angiosperm having two cotyledons.

Angiosperms encompass a variety of flowering plants, including, for example, cereal plants, leguminous plants, oilseed plants, trees, fruit-bearing plants and ornamental flowers, which general classes are not necessarily exclusive. Such angiosperms include for example, a cereal plant, which produces an edible grain cereal. Such cereal plants include, for example, corn, rice, wheat, barley, oat, rye, orchardgrass, guinea grass, sorghum and turfgrass. In addition, a leguminous plant is an angiosperm that is a member of the pea family (Fabaceae) and produces a characteristic fruit known as a legume. Examples of leguminous plants include, for example, soybean, pea, chickpea, moth bean, broad bean, kidney bean, lima bean, lentil, cowpea, dry bean, and peanut. Examples of legumes further also include alfalfa, birdsfoot trefoil, clover and sainfoin. Furthermore, an oilseed plant is an angiosperm that has seeds useful as a source of oil. Examples of oilseed plants include soybean, sunflower, rapeseed and cottonseed.

A tree is an angiosperm and is a perennial woody plant, generally with a single stem (trunk). Examples of trees include alder, ash, aspen, basswood (linden), beech, birch, cherry, cottonwood, elm, eucalyptus, hickory, locust, maple, oak, persimmon, poplar, sycamore, walnut and willows. Such trees are

used for pulp, paper, and structural material, as well as providing a major source of fuel.

A fruit-bearing plant also is an angiosperm and produces a mature, ripened ovary (usually containing seeds) that is suitable for human or animal consumption. Examples of fruit-bearing plants include grape, orange, lemon, grapefruit, avocado, date, peach, cherry, olive, plum, coconut, apple and pear trees and blackberry, blueberry, raspberry, strawberry, pineapple, tomato, cucumber and eggplant plants. An ornamental flower is an angiosperm cultivated for its decorative flower. Examples of ornamental flowers include rose, orchid, lily, tulip and chrysanthemum, snapdragon, camelia, carnation and petunia. The skilled artisan will recognize that the invention can be practiced on these or other angiosperms, as desired.

In various embodiments, the present invention provides a non-naturally occurring angiosperm having an ectopically expressible first nucleic acid molecule encoding a first floral meristem identity gene product, provided the first nucleic acid molecule is not ectopically expressed due to a mutation in an endogenous *TFL* gene. If desired, a non-naturally occurring angiosperm of the invention can contain an ectopically expressible second nucleic acid molecule encoding a second floral meristem identity gene product, which is different from the first floral meristem identity gene product.

An ectopically expressible nucleic acid molecule can be expressed, as desired, either constitutively or inducibly. Such an ectopically expressible nucleic acid molecule can be an endogenous nucleic acid molecule and can contain, for example, a mutation in its endogenous gene regulatory element or can contain an exogenous, heterologous gene regulatory element that is linked to and directs expression of the endogenous nucleic acid molecule. In addition, an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product can be an exogenous nucleic acid molecule encoding a floral meristem identity gene product and containing a heterologous gene regulatory element.

The invention provides, for example, a non-naturally occurring angiosperm containing a first ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product. If desired, a non-naturally occurring angiosperm of the invention can contain a floral meristem identity gene having a modified gene regulatory element and also can contain a second ectopically expressible nucleic acid molecule encoding a second floral meristem identity gene product, provided that neither the first nor second ectopically expressible nucleic acid molecule is ectopically expressed due to a mutation in an endogenous *TERMINAL FLOWER* gene.

As used herein, the term "modified gene regulatory element" means a regulatory element having a mutation that results in ectopic expression in shoot

meristem of the floral meristem identity gene regulated by the gene regulatory element. Such a gene regulatory element can be, for example, a promoter or enhancer element and can be positioned 5' or 3' to the coding sequence or within an intronic sequence of the floral meristem identity gene. Such a modification can be, for example, a nucleotide insertion, deletion or substitution and can be produced by chemical mutagenesis using a mutagen such as ethylmethane sulfonate (see Example IIIA) or by insertional mutagenesis using a transposable element. For example, a modified gene regulatory element can be a functionally inactivated binding site for TFL or a gene product regulated by TFL, such that modification of the gene regulatory element results in ectopic expression of the floral meristem identity gene product in shoot meristem.

The invention also provides a transgenic angiosperm containing a first exogenous gene promoter that regulates a first ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product and a second exogenous gene promoter that regulates a second ectopically expressible nucleic acid molecule encoding a second floral meristem identity gene product.

The invention also provides a transgenic angiosperm containing a first exogenous ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product and a second exogenous gene promoter that regulates a second ectopically

expressible nucleic acid molecule encoding a second floral meristem identity gene product, provided that the first nucleic acid molecule is not ectopically expressed due to a mutation in an endogenous *TERMINAL FLOWER* gene.

The invention also provides a transgenic angiosperm containing a first exogenous ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product and a second exogenous ectopically expressible nucleic acid molecule encoding a second floral meristem identity gene product, where the first floral meristem identity gene product is different from the second floral meristem identity gene product and provided that neither nucleic acid molecule is ectopically expressed due to a mutation in an endogenous *TERMINAL FLOWER* gene.

The ectopic expression of first and second floral meristem identity gene products can be particularly useful. For example, ectopic expression of AP1 and LFY in a plant promotes flowering earlier than ectopic expression of AP1 alone or ectopic expression of LFY alone. Thus, plant breeding, for example, can be further accelerated, if desired.

First and second floral meristem identity gene products can be, for example, AP1 and CAL, or can be AP1 and LFY or can be CAL and LFY. It should be recognized that where a transgenic angiosperm of the invention contains two exogenous nucleic acid molecules, the order of introducing such a first and a second nucleic acid

molecule is not important for purposes of the present invention. Thus, a transgenic angiosperm of the invention having, for example, AP1 as the first floral meristem identity gene product and CAL as the second floral meristem identity gene product is equivalent to a transgenic angiosperm having CAL as the first floral meristem identity gene product and AP1 as the second floral meristem identity gene product.

The invention also provides methods of converting shoot meristem to floral meristem in an angiosperm by ectopically expressing an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product in the angiosperm. Thus, the invention provides, for example, methods of converting shoot meristem to floral meristem in an angiosperm by introducing an exogenous ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product into the angiosperm, thereby producing a transgenic angiosperm. A floral meristem identity gene product such as AP1, CAL or LFY, or a chimeric protein containing, in part, a floral meristem identity gene product (see below) is useful in the methods of the invention.

As used herein, the term "introducing," when used in reference to an angiosperm, means transferring an exogenous nucleic acid molecule into the angiosperm. For example, an exogenous nucleic acid molecule can be introduced into an angiosperm by methods such as *Agrobacterium*-mediated transformation or direct gene

transf r methods including microprojectile-mediated transformation (Klein et al., Nature 327:70-73 (1987), which is incorporated herein by reference). These and other methods of introducing a nucleic acid molecule into an angiosperm are well known in the art (Bowman et al. (ed.), Arabidopsis: An Atlas of Morphology and Development, New York: Springer (1994); Valvekens et al., Proc. Natl. Acad. Sci., USA 85:5536-5540 (1988); and Wang et al., Transformation of Plants and Soil Microorganisms, Cambridge, UK: University Press (1995), each of which is incorporated herein by reference).

As used herein, the term "converting shoot meristem to floral meristem" means promoting the formation of flower progenitor tissue where shoot progenitor tissue would normally be formed. As a result of the conversion of shoot meristem to floral meristem, flowers form in an angiosperm where shoots normally would form. The conversion of shoot meristem to floral meristem can be identified using well known methods, such as scanning electron microscopy, light microscopy or visual inspection.

The invention also provides methods of converting shoot meristem to floral meristem in an angiosperm by introducing a first ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product and a second ectopically expressible nucleic acid molecule encoding a second floral meristem identity gene product into the angiosperm. As discussed above, first and second floral

meristem identity gene products useful in the invention can be, for example, AP1 and CAL or AP1 and LFY or CAL and LFY.

The invention also provides methods of promoting early flowering in an angiosperm by ectopically expressing a nucleic acid molecule encoding a floral meristem identity gene product in the angiosperm, provided that the nucleic acid molecule is not ectopically expressed due to a mutation in an endogenous *TERMINAL FLOWER* gene. For example, the invention provides methods of promoting early flowering in an angiosperm by introducing an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product into the angiosperm, thus producing a transgenic angiosperm. A floral meristem identity gene product such as AP1, CAL or LFY, or a chimeric protein containing, in part, a floral meristem identity gene product (see below) is useful in methods of promoting early flowering.

The present invention further provides nucleic acid molecules encoding floral meristem identity gene products. For example, the invention provides a nucleic acid molecule encoding CAL, having at least about 70 percent amino acid identity with amino acids 1 to 160 of SEQ ID NO: 10 or SEQ ID NO: 11. The invention also provides a nucleic acid molecule encoding *Arabidopsis thaliana* CAL having the amino acid sequence shown in Figure 5 (SEQ ID NO: 10) and a nucleic acid molecule encoding *Brassica oleracea* CAL having the amino acid

sequence shown in Figure 6 (SEQ ID NO: 12). In addition, the invention provides a nucleic acid molecule encoding *Brassica oleracea* AP1 having the amino acid sequence shown in Figure 2 (SEQ ID NO: 4) and a nucleic acid molecule encoding *Brassica oleracea* var. *botrytis* AP1 having the amino acid sequence shown in Figure 3 (SEQ ID NO: 6). The invention also provides a nucleic acid molecule encoding *Zea mays* AP1 having the amino acid sequence shown in Figure 4 (SEQ ID NO: 8).

As disclosed herein, CAL is highly conserved among different angiosperms. For example, *Arabidopsis* CAL (SEQ ID NO: 10) and *Brassica oleracea* CAL (SEQ ID NO: 12) share about 80 percent amino acid identity. In the region from amino acid 1 to amino acid 160, *Arabidopsis* CAL and *Brassica oleracea* CAL are about 89 percent identical at the amino acid level. Using a nucleotide sequence derived from a conserved region of SEQ ID NO: 9 or SEQ ID NO: 11, a nucleic acid molecule encoding a novel CAL ortholog can be isolated from other angiosperms. Using methods such as those described by Purugganan et al. (Genetics 40: 345-356 (1995)), one can readily confirm that the newly isolated molecule is a CAL ortholog. Thus, a nucleic acid molecule encoding CAL, which has at least about 70 percent amino acid identity with *Arabidopsis* CAL (SEQ ID NO: 10) or *Brassica oleracea* CAL (SEQ ID NO: 12), can be isolated and identified using well known methods.

The invention also provides a nucleic acid molecul encoding a truncated CAL gene product. For

example, the invention provides a nucleic acid molecule encoding the *Brassica oleracea* var. *botrytis* CAL gene product (BobCAL). BobCAL contains 150 amino acids of the approximately 255 amino acids encoded by a full-length CAL cDNA (see Figure 7; SEQ ID NO: 14; see, also, Figure 8B).

The invention also provides a nucleic acid containing the *Arabidopsis thaliana AP1* gene (Figure 10; SEQ ID NO: 17), a nucleic acid molecule containing the *Brassica oleracea AP1* gene (Figure 11; SEQ ID NO: 18) and a nucleic acid molecule containing the *Brassica oleracea var. botrytis AP1* gene (Figure 12; SEQ ID NO: 19). In addition, the invention also provides a nucleic acid containing the *Arabidopsis thaliana CAL* gene (Figure 13; SEQ ID NO: 20) and a nucleic acid molecule containing the *Brassica oleracea CAL* gene (Figure 11; SEQ ID NO: 21). In addition, the invention provides a nucleic acid molecule containing the *Brassica oleracea var. botrytis CAL* gene (Figure 15; SEQ ID NO: 22).

The invention further provides a nucleotide sequence that hybridizes under relatively stringent conditions to a nucleic acid molecule encoding a CAL, or a complementary sequence thereof. In particular, such a nucleotide sequence can hybridize under relatively stringent conditions to a nucleic acid molecule encoding *Arabidopsis CAL* (SEQ ID NO: 9) or *Brassica oleracea CAL* (SEQ ID NO: 11), or a complementary sequence thereof. Similarly, the present invention provides a nucleotide sequence that hybridizes under relatively stringent

conditions to a nucleic acid molecule encoding *Zea mays* AP1 (SEQ ID NO: 7), or a complementary sequence thereof.

In general, a nucleotide sequence that hybridizes under relatively stringent conditions to a nucleic acid molecule is a single-stranded nucleic acid sequence that can range in size from about 10 nucleotides to the full-length of a gene or a cDNA. Such a nucleotide sequence can be chemically synthesized, using routine methods or can be purchased from a commercial source. In addition, such nucleotide sequences can be obtained by enzymatic methods such as random priming methods, the polymerase chain reaction (PCR) or by standard restriction endonuclease digestion, followed by denaturation (Sambrook et al., *supra*, 1989).

A nucleotide sequence that hybridizes under relatively stringent conditions to a nucleic acid molecule can be used, for example, as a primer for PCR (Innis et al. (ed.) PCR Protocols: A Guide to Methods and Applications, San Diego, CA: Academic Press, Inc. (1990)). Such a nucleotide sequence generally contains about 10 to about 50 nucleotides.

A nucleotide sequence that hybridizes under relatively stringent conditions to a nucleic acid molecule also can be used to screen a cDNA or genomic library to obtain a related nucleotide sequence. For example, a cDNA library that is prepared from rice or wheat can be screened with a nucleotide sequence derived from the *Zea mays* AP1 sequence in order to isolate a rice

or wheat ortholog of AP1. Generally, such a nucleotide sequence contains at least about 14-16 nucleotides depending, for example, on the hybridization conditions to be used.

A nucleotide sequence derived from a nucleic acid molecule encoding *Zea mays* AP1 (SEQ ID NO: 7) also can be used to screen a *Zea mays* cDNA library to isolate a sequence that is related to but distinct from AP1. Furthermore, such a hybridizing nucleotide sequence can be used to analyze RNA levels or patterns of expression, as by northern blotting or by *in situ* hybridization to a tissue section. Such a nucleotide sequence also can be used in Southern blot analysis to evaluate gene structure and identify the presence of related gene sequences.

One skilled in the art would select a particular nucleotide sequence that hybridizes under relatively stringent conditions to a nucleic acid molecule encoding a floral meristem identity gene product based on the application for which the sequence will be used. For example, in order to isolate an ortholog of *AP1*, one can choose a region of *AP1* that is highly conserved among known *AP1* sequences such as *Arabidopsis AP1* (SEQ ID NO: 1) and *Zea mays AP1* (GenBank accession number L46400; SEQ ID NO: 7). Similarly, in order to isolate an ortholog of *CAL*, one can choose a region of *CAL* that is highly conserved among known *CAL* cDNAs, such as *Arabidopsis CAL* (SEQ ID NO: 9) and *Brassica CAL* (SEQ ID NO: 11). It further would be recognized, for example, that the region encoding the MADS domain, which is common

to a number of genes, can be excluded from the nucleotide sequence. In addition, one can use a full-length *Arabidopsis AP1* or *CAL* cDNA nucleotide sequence (SEQ ID NO: 1 or SEQ ID NO: 9) to isolate an ortholog of *AP1* or *CAL*.

For example, the *Arabidopsis AP1* cDNA shown in Figure 1 (SEQ ID NO: 1) can be used as a probe to identify and isolate a novel *AP1* ortholog. Similarly, the *Arabidopsis CAL* cDNA shown in Figure 5 (SEQ ID NO: 9) can be used to identify and isolate a novel *CAL* ortholog (see Examples IA and IIIC, respectively). In order to identify related MADS domain genes, a nucleotide sequence derived from the MADS domain of *AP1* or *CAL*, for example, also can be useful to isolate a related gene sequence encoding this DNA-binding motif.

Hybridization utilizing a nucleotide sequence of the invention requires that hybridization be performed under relatively stringent conditions such that non-specific hybridization is minimized. Appropriate hybridization conditions can be determined empirically, or can be estimated based, for example, on the relative G+C content of the probe and the number of mismatches between the probe and target sequence, if known. Hybridization conditions can be adjusted as desired by varying, for example, the temperature of hybridizing or the salt concentration (Sambrook, *supra*, 1989).

The invention also provides a vector containing a nucleic acid molecule encoding a CAL gene product. In

addition, the invention provides a vector containing a nucleic acid molecule encoding the *Zea mays* AP1 gene product. A vector can be a cloning vector or an expression vector and provides a means to transfer an exogenous nucleic acid molecule into a host cell, which can be a prokaryotic or eukaryotic cell. Such vectors are well known and include plasmids, phage vectors and viral vectors. Various vectors and methods for introducing such vectors into a cell are described, for example, by Sambrook et al., *supra*, 1989, and by Glick and Thompson (eds.), Methods in Plant Molecular Biology and Biotechnology, Boca Raton, FL: CRC Press (1993), which is incorporated herein by reference.

The invention also provides an expression vector containing a nucleic acid molecule encoding a floral meristem identity gene product such as CAL, AP1 or LFY. Expression vectors are well known in the art and provide a means to transfer and express an exogenous nucleic acid molecule into a host cell. Thus, an expression vector contains, for example, transcription start and stop sites such as a TATA sequence and a poly-A signal sequence, as well as a translation start site such as a ribosome binding site and a stop codon, if not present in the coding sequence.

An expression vector can contain, for example, a constitutive regulatory element useful for promoting expression of an exogenous nucleic acid molecule in a plant cell. The use of a constitutive regulatory element can be particularly advantageous because expression from

the element is relativ ly independent of developmentally regulated or tissue-specific factors. For example, the cauliflower mosaic virus 35S promoter (CaMV35S) is a well-characterized constitutive regulatory element that produces a high level of expression in all plant tissues (Odell et al., Nature 313:810-812 (1985), which is incorporated herein by reference). The CaMV35S promoter is particularly useful because it is active in numerous different angiosperms (Benfey and Chua, Science 250:959-966 (1990), which is incorporated herein by reference; Odell et al., *supra*, 1985). Other constitutive regulatory elements useful for expression in an angiosperm include, for example, the nopaline synthase (*nos*) gene promoter (An, Plant Physiol. 81:86 (1986), which is herein incorporated by reference).

In addition, an expression vector of the invention can contain a regulated gene regulatory element such as a promoter or enhancer element. A particularly useful regulated promoter is a tissue-specific promoter such as the shoot meristem-specific *CDC2* promoter (Hemerly et al., Plant Cell 5:1711-1723 (1993), which is incorporated herein by reference), or the *AGL8* promoter, which is active in the apical shoot meristem immediately after the transition to flowering (Mandel and Yanofsky, Plant Cell 7:1763-1771 (1995), which is incorporated herein by reference).

An expression vector of the invention also can contain an inducible regulatory element, which has conditional activity dependent upon the presence of a

particular regulatory factor. Useful inducible regulatory elements include, for example, a heat-shock promoter (Ainley and Key, Plant Mol. Biol. 14:949 (1990), which is herein incorporated by reference) or a nitrate-inducible promoter derived from the spinach nitrite reductase gene (Back et al., Plant Mol. Biol. 17:9 (1991), which is herein incorporated by reference). A hormone-inducible element (Yamaguchi-Shinozaki et al., Plant Mol. Biol. 15:905 (1990) and Kares et al., Plant Mol. Biol. 15:225 (1990), which are herein incorporated by reference) or a light-inducible promoter, such as that associated with the small subunit of RuBP carboxylase or the LHCP gene families (Feinbaum et al., Mol. Gen. Genet. 226:449 (1991) and Lam and Chua, Science 248:471 (1990), which are herein incorporated by reference) also can be useful in an expression vector of the invention. A human glucocorticoid response element also can be used to achieve steroid hormone-dependent gene expression in plants (Schena et al., Proc. Natl. Acad. Sci. USA 88:10421 (1991), which is herein incorporated by reference).

An appropriate gene regulatory element such as a promotor is selected depending on the desired pattern or level of expression of a nucleic acid molecule linked thereto. For example, a constitutive promoter, which is active in all tissues, would be appropriate to express a desired gene product in all cells containing the vector. In addition, it can be desirable to restrict expression of a nucleic acid molecule to a particular tissue or

during a particular stage of development. A developmentally regulated or tissue-specific expression can be useful for this purpose and can avoid potential undesirable side-effects that can accompany unregulated expression. Inducible expression also can be particularly useful to manipulate the timing of gene expression such that, for example, a population of transgenic angiosperms of the invention that contain an expression vector comprising a floral meristem identity gene linked to an inducible promoter can be induced to flower essentially at the same time. Such timing of flowering can be useful, for example, for manipulating the time of crop harvest.

The invention also provides a kit containing an expression vector having a nucleic acid molecule encoding a floral meristem identity gene product. Such a kit is useful for converting shoot meristem to floral meristem in an angiosperm or for promoting early flowering in an angiosperm. If desired, such a kit can contain appropriate reagents, which can allow relatively high efficiency of transformation of an angiosperm with the vector. Furthermore, a control plasmid lacking the floral meristem identity gene can be included in the kit to determine, for example, the efficiency of transformation.

The invention further provides a host cell containing a vector comprising a nucleic acid molecule encoding CAL. A host cell can be prokaryotic or eukaryotic and can be, for example, a bacterial cell,

yeast cell, insect cell, xenopus cell, mammalian cell or plant cell.

The invention also provides a transgenic garden variety cauliflower plant containing an exogenous nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a CAL gene product and a nucleic acid molecule encoding an AP1 gene product. Such a transgenic cauliflower plant can produce an edible flower in place of the typical cauliflower vegetable.

A nucleic acid encoding CAL has been isolated from a *Brassica oleracea* line that produces wild-type flowers (*BoCAL*) and from the common garden variety of cauliflower, *Brassica oleracea* var. *botrytis* (*BobCAL*), which lacks flowers. The *Brassica oleracea CAL* cDNA (SEQ ID NO: 10) is highly similar to the *Arabidopsis CAL* cDNA (SEQ ID NO: 12; and see Figure 8). In contrast, the *Brassica oleracea* var. *botrytis CAL* cDNA contains a stop codon, predicting that the BobCAL protein will be truncated after amino acid 150 (SEQ ID NO: 14 and see Figure 8). The correlation of full-length *Arabidopsis* and *Brassica oleracea* CAL gene products with a flowering phenotype indicates that transformation of non-flowering garden varieties of cauliflower such as *Brassica oleracea* var. *botrytis* with a full-length *CAL* cDNA can induce flowering in the transgenic cauliflower plant.

As used herein, the term "CAL gene product" means a full-length CAL gene product that does not terminate substantially before amino acid 255 and that,

when ectopically expressed in shoot meristem, converts shoot meristem to floral meristem. A nucleic acid molecule encoding a CAULIFLOWER gene product can be, for example, a nucleic acid molecule encoding *Arabidopsis* CAL shown in Figure 5 (SEQ ID NO: 9) or a nucleic acid molecule encoding *Brassica oleracea* CAL shown in Figure 6 (SEQ ID NO: 11). In comparison, a nucleic acid molecule encoding a truncated CAL gene product that terminates substantially before amino acid 255, such as the encoded truncated BobCAL gene product (SEQ ID NO: 13), is not a nucleic acid molecule encoding a CAL gene product as defined herein. Furthermore, ectopic expression of BobCAL in an angiosperm does not result in conversion of shoot meristem to floral meristem.

As used herein, the term "AP1 gene product" means a full-length AP1 gene product that does not terminate substantially before amino acid 256. A nucleic acid molecule encoding an AP1 gene product can be, for example, a nucleic acid molecule encoding *Arabidopsis* AP1 shown in Figure 1 (SEQ ID NO: 1), *Brassica oleracea* AP1 shown in Figure 2, (SEQ ID NO: 3), *Brassica oleracea* var. *botrytis* AP1 shown in Figure 3 (SEQ ID NO: 5) or *Zea mays* AP1 shown in Figure 4 (SEQ ID NO: 7).

The invention provides a CAL polypeptide having at least about 70 percent amino acid identity with amino acids 1 to 160 of SEQ ID NO: 10 or SEQ ID NO: 12. For example, the *Arabidopsis thaliana* CAL polypeptide, having the amino acid sequence shown as amino acids 1 to 255 in Figure 5 (SEQ ID NO: 10), and the *Brassica oleracea* CAL

polypeptide, having the amino acid sequence shown as amino acids 1 to 255 in Figure 6 (SEQ ID NO: 12) are provided by the invention.

The invention also provides the truncated *Brassica oleracea var. botrytis* CAL polypeptide having the amino acid sequence shown as amino acids 1 to 150 in Figure 7 (SEQ ID NO: 14). The BobCAL polypeptide can be useful as an immunogen to produce an antibody that specifically binds the truncated BoCAL polypeptide, but does not bind a full length CAL gene product. Such an antibody can be useful to distinguish between a full length CAL and truncated CAL.

The invention provides also provides a *Zea mays* AP1 polypeptide. As used herein, the term "polypeptide" is used in its broadest sense to include proteins, polypeptides and peptides, which are related in that each consists of a sequence of amino acids joined by peptide bonds. For convenience, the terms "polypeptide," "protein" and "gene product" are used interchangeably. While no specific attempt is made to distinguish the size limitations of a protein and a peptide, one skilled in the art would understand that proteins generally consist of at least about 50 to 100 amino acids and that peptides generally consist of at least two amino acids up to a few dozen amino acids. The term polypeptide is used generally herein to include any such amino acid sequence.

The term polypeptide also includes an active fragment of a floral meristem identity gene product. As

used herein, the term "active fragment," means a polypeptide portion of a floral meristem identity gene product that can convert shoot meristem to floral meristem or can provide early flowering. For example, an active fragment of a CAL polypeptide can consist of an amino acid sequence derived from a CAL protein as shown in Figure 5 or 6 (SEQ ID NOS: 10 and 12) and that has an activity of a CAL. An active fragment can be, for example, an amino terminal or carboxyl terminal truncated form of *Arabidopsis thaliana* CAL or *Brassica oleracea* CAL (SEQ ID NOS: 10 or 12, respectively). Such an active fragment can be produced using well known recombinant DNA methods (Sambrook et al., *supra*, 1989). The product of the *BobCAL* gene, which is truncated at amino acid 150, lacks activity in converting shoot meristem to floral meristem and, therefore, is an example of a polypeptide portion of a CAL floral meristem identity gene product that is not an "active fragment."

An active fragment of a floral meristem identity gene product can convert shoot meristem to floral meristem and is readily identified using the methods described in Example II, below). Briefly, *Arabidopsis* can be transformed with a nucleic acid molecule encoding a portion of a floral meristem identity gene product, in order to determine whether the fragment can convert shoot meristem to floral meristem or promote early flowering and, therefore, has an activity of a floral meristem identity gene product.

The invention further provides an antibody that specifically binds a CAL polypeptide, an antibody that specifically binds the truncated *Brassica oleracea* var. *botrytis* CAL polypeptide, and an antibody that specifically binds the *Zea mays* AP1 polypeptide. As used herein, the term "antibody" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as polypeptide fragments of antibodies that retain a specific binding activity for CAL protein of at least about 1×10^5 M^{-1}. One skilled in the art would know that anti-CAL antibody fragments such as Fab, $F(ab')_2$ and Fv fragments can retain specific binding activity for CAL and, thus, are included within the definition of an antibody. In addition, the term "antibody" as used herein includes naturally occurring antibodies as well as non-naturally occurring antibodies and fragments that have binding activity such as chimeric antibodies or humanized antibodies. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, produced recombinantly or obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Huse et al., Science 246:1275-1281 (1989), which is incorporated herein by reference.

An antibody "specific for" a polypeptide, or that "specifically binds" a polypeptide, binds with substantially higher affinity to that polypeptide than to an unrelated polypeptide. An antibody specific for a polypeptide also can have specificity for a related polypeptide. For example, an antibody specific for

Arabidopsis CAL also can have specificity for *Brassica oleracea* CAL.

An anti-CAL antibody, for example, can be prepared using a CAL fusion protein or a synthetic peptide encoding a portion of *Arabidopsis* CAL or of *Brassica oleracea* CAL as an immunogen. One skilled in the art would know that purified CAL protein, which can be prepared from natural sources or produced recombinantly, or fragments of CAL, including a peptide portion of CAL such as a synthetic peptide, can be used as an immunogen. Non-immunogenic fragments or synthetic peptides of CAL can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). In addition, various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art and described, for example, by Harlow and Lane, Antibodies: A laboratory manual (Cold Spring Harbor Laboratory Press, 1988), which is incorporated herein by reference. An antibody that specifically binds the truncated Bob CAL polypeptide or an antibody that specifically binds the *Zea mays* AP1 polypeptide similarly can be produced using such methods. An antibody that specifically binds the truncated *Brassica oleracea* var. *botrytis* CAL polypeptide can be particularly useful to distinguish between full-length CAL polypeptide and truncated CAL polypeptide.

The invention provides a method of identifying a *Brassica* having a modified *CAL* allele by detecting a polymorphism associated with a *CAL* locus, where the *CAL* locus comprises a modified *CAL* allele that does not encode an active CAL gene product. Such a method is useful for the genetic improvement of *Brassica* plants, a genus of great economic value.

Brassica plants are a highly diverse group of crop plants useful as vegetables and as sources of condiment mustard, edible and industrial oil, animal fodder and green manure. *Brassica* crops encompass a variety of well known vegetables including cabbage, cauliflower, broccoli, collard, kale, mustard greens, Chinese cabbage and turnip, which can be interbred for crop improvement (see, for example, King, Euphytica 50:97-112 (1990) and Crisp and Tapsell, Genetic improvement of vegetable crops pp. 157-178 (1993), each of which is herein incorporated by reference).

Breeding of *Brassica* crops is useful, for example, for improving the quality and early development of vegetables. In addition, such breeding can be useful to increase disease resistance, such as resistance, of a *Brassica* to clubroot disease or mildew; viral resistance, such as resistance to turnip mosaic virus and cauliflower mosaic virus; or pest resistance (King, *supra*, 1990).

The use of polymorphic molecular markers in the breeding of *Brassicae* is well recognized in the art (Crisp and Tapsell, *supra*, 1993). Identification of a

polymorphic molecular marker that is associated with a desirable trait can vastly accelerate the time required to breed the desirable trait into a new *Brassica* species or variant. In particular, since many rounds of backcrossing are required to breed a new trait into a different genetic background, early detection of a desirable trait by molecular methods can be performed prior to the time a plant is fully mature, thus accelerating the rate of crop breeding (see, for example, Figidore et al., Euphytica 69: 33-44 (1993), which is herein incorporated by reference).

A polymorphism associated with a *CAL* locus comprising a modified *CAL* allele that does not encode an active CAL gene product, is disclosed herein. Figure 6 shows the nucleotide (SEQ ID NO: 11) and amino acid (SEQ ID NO: 12) sequence of *Brassica oleracea* CAL (BoCAL), and Figure 7 shows the nucleotide (SEQ ID NO: 13) and amino acid (SEQ ID NO: 14) sequence of *Brassica oleracea* var. *botrytis* CAL (BobCAL). At amino acid 150, which is glutamic acid (Glu) in BoCAL, a stop codon is present in BobCAL. This polymorphism results in a truncated BobCAL gene product that is not active as a floral meristem identity gene product. The *BoCAL* nucleic acid sequence (ACGAGT) can be readily distinguished from the *BobCAL* nucleic acid sequence (ACTAGT) using well known molecular methods. For example, the polymorphic ACTAGT *BobCAL* sequence is recognized by a SpeI restriction endonuclease site, whereas the ACGAGT *BoCAL* sequence is not recognized by SpeI. Thus, a restriction fragment length polymorphism (RFLP) in BobCAL provides a simple means for

identifying a modified *CAL* allele (*BobCAL*) and, therefore, can serve as a marker to predict the inheritance of the "cauliflower" phenotype.

A modified *CAL* allele encoding a truncated CAL gene product also can serve as a marker to predict the "cauliflower" phenotype in other cauliflower variants. For example, nine *romanesco* variants of *Brassica oleracea* var. *botrytis*, which each have the "cauliflower" phenotype, were examined for the presence of a stop codon at position 151 of the CAL coding sequence. All nine of the *romanesco* variants contained the SpeI site that indicates a stop codon and, thus, a truncated CAL gene product. In contrast, *Brassica oleracea* variants that lack the "cauliflower" phenotype (broccoli and brussels sprouts) were examined for the SpeI site. In every case, the broccoli and brussel sprout variants had a full-length CAL coding sequence, as indicated by the absence of the distinguishing SpeI site. Thus, a truncated CAL gene product can be involved in the "cauliflower phenotype" in numerous different *Brassica* variants.

As used herein, the term "modified *CAL* allele" means a *CAL* allele that does not encode a CAL gene product active in converting shoot meristem to floral meristem. A modified *CAL* allele can have a modification within a gene regulatory element such that a CAL gene product is not produced. In addition, a modified *CAL* allele can have a modification such as a mutation, deletion or insertion in a *CAL* coding sequence which

results in an inactive CAL gene product. For example, an inactive CAL gene product can result from a mutation creating a stop codon, such that a truncated, inactive CAL gene product lacking the ability to convert shoot meristem to floral meristem is produced.

As used herein, the term "associated" means closely linked and describes the tendency of two genetic loci to be inherited together as a result of their proximity. If two genetic loci are associated and are polymorphic, one locus can serve as a marker for the inheritance of the second locus. Thus, a polymorphism associated with a *CAL* locus comprising a modified *CAL* allele can serve as a marker for inheritance of the modified *CAL* allele. An associated polymorphism can be located in proximity to a *CAL* gene or can be located within a *CAL* gene.

A polymorphism in a nucleic acid sequence can be detected by a variety of methods. For example, if the polymorphism occurs in a particular restriction endonuclease site, the polymorphism can be detected by a difference in restriction fragment length observed following restriction with the particular restriction endonuclease and hybridization with a nucleotide sequence that is complementary to a nucleic acid sequence including a polymorphism.

The use of restriction fragment length polymorphism as an aid to breeding *Brassicae* is well known in the art (see, for example, Slocum et al., Theor.

Appl. Genet. 80:57-64 (1990); Kennard et al., Theor. Appl. Genet. 87:721-732 (1994); and Figidore et al., *supra*, 1993, each of which is herein incorporated by reference). A restriction endonuclease such as SpeI, which is useful for identifying the presence of a BobCAL allele in an angiosperm, is readily available and can be purchased from a commercial source. Furthermore, a nucleotide sequence that is complementary to a nucleic acid sequence having a polymorphism associated with a *CAL* locus comprising a modified *CAL* allele can be derived, for example, from the nucleic acid molecule encoding *Brassica oleracea* var. *botrytis* CAL shown in Figure 7 (SEQ ID NO: 13) or from the nucleic acid molecule encoding *Brassica oleracea CAL* shown in Figure 6 (SEQ ID NO: 11).

In some cases, a polymorphism is not distinguishable by a RFLP, but nevertheless can be used to identify a *Brassica* having a modified *CAL* allele. For example, the polymerase chain reaction (PCR) can be used to detect a polymorphism associated with a *CAL* locus comprising a modified *CAL* allele. Specifically, a polymorphic region of a modified allele can be selectively amplified by using a primer that matches the nucleotide sequence of one allele of a polymorphic locus, but does not match the sequence of the second allele (Sobral and Honeycutt, The Polymerase Chain Reaction, pp. 304-319 (1994), which is herein incorporated by reference). Other well-known approaches for analyzing a polymorphism using PCR include discriminant hybridization of PCR-amplified DNA to allele-specific oligonucleotides

and denaturing gradient gel electrophoresis (s e Innis et al., *supra*, 1990).

The invention further provides a nucleic acid molecule encoding a chimeric protein, comprising a nucleic acid molecule encoding a floral meristem identity gene product such as AP1, LFY or CAL operably linked to a nucleic acid molecule encoding a ligand binding domain. Expression of a chimeric protein of the invention in an angiosperm is particularly useful because the ligand binding domain confers regulatable activity on a gene product such as a floral meristem identity gene product to which it is fused. Specifically, the floral meristem identity gene product component of the chimeric protein is inactive in the absence of the particular ligand, whereas, in the presence of ligand, the ligand binds the ligand binding domain, resulting in floral meristem identity gene product activity.

A nucleic acid molecule encoding a chimeric protein of the invention contains a nucleic acid molecule encoding a floral meristem identity gene product, such as a nucleic acid molecule encoding the amino acid sequence shown in Figure 1 (SEQ ID NO: 2), in Figure 5 (SEQ ID NO: 10), or in Figure 9 (SEQ ID NO: 10), either of which is operably linked to a nucleic acid molecule encoding a ligand binding domain. The expression of such a nucleic acid molecule results in the production of a chimeric protein comprising a floral meristem identity gene product fused to a ligand binding domain. Thus, the invention also provides a chimeric protein comprising a

floral meristem identity gene product fused to a ligand binding domain.

A ligand binding domain useful in a chimeric protein of the invention can be a steroid binding domain such as the ligand binding domain of a glucocorticoid receptor, estrogen receptor, progesterone receptor, androgen receptor, thyroid receptor, vitamin D receptor or retinoic acid receptor. A particularly useful ligand binding domain is a glucocorticoid receptor ligand binding domain, encompassed, for example, within amino acids 512 to 795 of the rat glucocorticoid receptor as shown in Figure 16 (SEQ ID NO: 24; Miesfeld et al., Cell 46:389-399 (1986), which is incorporated herein by reference).

A chimeric protein containing a ligand binding domain, such as the rat glucocorticoid receptor ligand binding domain, confers glucocorticoid-dependent activity on the chimeric protein. For example, the activity of chimeric proteins consisting of adenovirus E1A, c-myc, c-fos, the HIV-1 Rev transactivator, MyoD or maize regulatory factor R fused to the rat glucocorticoid receptor ligand binding domain is regulated by glucocorticoid hormone (Eilers et al., Nature 340:66 (1989); Superti-Furga et al., Proc. Natl. Acad. Sci., U.S.A. 88:5114 (1991); Hope et al., Proc. Natl. Acad. Sci., U.S.A. 87:7787 (1990); Hollenberg et al., Proc. Natl. Acad. Sci., U.S.A. 90:8028 (1993), each of which is incorporated herein by reference).

Such a chimeric protein also can be regulated in plants. For example, a chimeric protein containing a heterologous protein fused to a rat glucocorticoid receptor ligand binding domain (amino acids 512 to 795) was expressed under the control of the constitutive cauliflower mosaic virus 35S promoter in *Arabidopsis*. The activity of the chimeric protein was inducible; the chimeric protein was inactive in the absence of ligand, and became active upon treatment of transformed plants with a synthetic glucocorticoid, dexamethasone (Lloyd et al., Science 266:436-439 (1994), which is incorporated herein by reference). As disclosed herein, a ligand binding domain fused to a floral meristem identity gene product can confer ligand inducibility on the activity of a fused floral meristem identity gene product in plants such that, upon exposure to a particular ligand, the floral meristem identity gene product is active.

Methods for constructing a nucleic acid molecule encoding a chimeric protein are routine and well known in the art (Sambrook et al., *supra*, 1989). For example, the skilled artisan would recognize that a stop codon in the 5' nucleic acid molecule must be removed and that the two nucleic acid molecules must be linked such that the reading frame of the 3' nucleic acid molecule is preserved. Methods of transforming plants with nucleic acid molecules also are well known in the art (see, for example, Mohoney et al., U.S. patent number 5,463,174, and Barry et al., U.S. patent number 5,463,175, each of which is incorporated herein by reference).

As used herein, the term "operably linked," when used in reference to two nucleic acid molecules comprising a nucleic acid molecule encoding a chimeric protein, means that the two nucleic acid molecules are linked in frame such that a full-length chimeric protein can be expressed. In particular, the 5' nucleic acid molecule, which encodes the amino-terminal portion of the chimeric protein, must be linked to the 3' nucleic acid molecule, which encodes the carboxyl-terminal portion of the chimeric protein, such that the carboxyl-terminal portion of the chimeric protein is produced in the correct reading frame.

The invention further provides a transgenic angiosperm containing a nucleic acid molecule encoding a chimeric protein, comprising a nucleic acid molecule encoding a floral meristem identity gene product such as AP1, CAL or LFY linked to a nucleic acid molecule encoding a ligand binding domain. Such a transgenic angiosperm is particularly useful because the angiosperm can be induced to flower by contacting the angiosperm with a ligand that binds the ligand binding domain. Thus, the invention provides a method of promoting early flowering or of converting shoot meristem to floral meristem in a transgenic angiosperm containing a nucleic acid molecule encoding a chimeric protein of the invention, comprising expressing the nucleic acid molecule encoding the chimeric protein in the angiosperm, and contacting the angiosperm with a ligand that binds the ligand binding domain, wherein binding of the ligand to the ligand binding domain activates the floral

meristem identity gene product. In particular, the invention provides methods of promoting early flowering or of converting shoot meristem to floral meristem in a transgenic angiosperm containing a nucleic acid molecule encoding a chimeric protein that consists of a nucleic acid molecule encoding AP1 or CAL or LFY linked to a nucleic acid molecule encoding a glucocorticoid receptor ligand binding domain by contacting the transgenic angiosperm with a glucocorticoid such as dexamethasone.

As used herein, the term "ligand" means a naturally occurring or synthetic chemical or biological molecule such as a simple or complex organic molecule, a peptide, a protein or an oligonucleotide that specifically binds a ligand binding domain. A ligand of the invention can be used, alone, in solution or can be used in conjunction with an acceptable carrier that can serve to stabilize the ligand or promote absorption of the ligand by an angiosperm.

One skilled in the art can readily determine the optimum concentration of ligand needed to bind a ligand binding domain and render a floral meristem identity gene product active. Generally, a concentration of about 1 nM to 1μM dexamethasone is useful for activating floral meristem identity gene product activity in a chimeric protein comprising a floral meristem identity gene product and a glucocorticoid receptor ligand binding domain (Lloyd et al., *supra*, 1994).

A transgenic angiosperm expressing a chimeric protein of the invention can be contacted with ligand in a variety of manners including, for example, by spraying, injecting or immersing the angiosperm. Further, a plant may be contacted with a ligand by adding the ligand to the plant's water supply or to the soil, whereby the ligand is absorbed into the angiosperm.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

Identification and characterization of the *Zea mays APETALA1* cDNA

This example describes the isolation and characterization of the *Zea mays* ZAP-1 "gene", which is an ortholog of the Arabidopsis floral meristem identity gene, *AP1*.

A. Identification and characterization of a nucleic acid sequence encoding ZAP-1

The utility of using a cloned floral homeotic gene from *Arabidopsis* to identify the putative ortholog in maize has previously been demonstrated (Schmidt et al., *supra*, (1993), which is incorporated herein by reference). As described in Mena et al. (Plant J. 8(6):845-854 (1995)), the maize ortholog of the *Arabidopsis AP1* floral meristem identity gene, was isolated by screening a *Zea mays* ear cDNA library using

the *Arabidopsis AP1* cDNA (SEQ ID NO: 1) as a probe. A cDNA library was prepared from wild-type immature ears as described by Schmidt et al., *supra*, 1993, using an *Arabidopsis AP1* cDNA sequence as a probe. The *Arabidopsis AP1* cDNA (SEQ ID NO: 1), which is shown in Figure 1 (SEQ ID NO 1), was used as the probe. Low-stringency hybridizations with the AP1 probe were conducted as described previously for the isolation of *ZAG1* using the *AG* cDNA as a probe (Schmidt et al., *supra*, 1993). Positive plaques were isolated and cDNAs were recovered in Bluescript by *in vivo* excision. Double-stranded sequencing was performed using the Sequenase Version 2.0 kit (U.S. Biochemical, Cleveland, Ohio) according to the manufacturer's protocol.

The cDNA sequence and deduced amino acid sequence for *ZAP1* are shown in Figure 4 (SEQ ID NOS: 7 and 8). The deduced amino acid sequence for *ZAP1* shares 89% identity with *Arabidopsis AP1* through the MADS domain (amino acids 1 to 57) and 70% identity through the first 160 amino acids, which includes the K domain. The high level of amino acid sequence identity between *ZAP1* and *AP1* (SEQ ID NOS: 8 and 2), as well as the expression pattern of *ZAP1* in maize florets (see below), indicates that *ZAP1* is the maize ortholog of *Arabidopsis AP1*.

B. RNA expression pattern of ZAP1

Total RNA was isolated from different maize tissues as described by Cone et al., Proc. Natl. Acad. Sci., USA 83:9631-9635 (1986), which is herein

incorporated by reference. RNA was prepared from ears or tassels at early developing stages (approximately 2 cm in size), husk leaves from developing ear shoots, shoots and roots of germinated seedlings, leaves from 2 to 3 week old plants and endosperm, and embryos at 18 days after pollination. Mature floral organs were dissected from ears at the time of silk emergence or from tassels at several days pre-emergence. To study expression patterns in the mature female flower, carpels were isolated and the remaining sterile organs were pooled and analyzed together. In the same way, stamens were dissected and collected from male florets and the remaining organs (excluding the glumes) were pooled as one sample.

RNA concentration and purity was determined by absorbance at 260/280 nM, and equal amounts (10 μg) were fractionated on formaldehyde-agarose gels. Gels were stained in a solution of 0.125 μg ml^{-1} acridine orange to confirm the integrity of the RNA samples and the uniformity of gel loading, then RNA was blotted on to Hybond-N® membranes (Amersham International, Arlington Heights, Illinois) according to the manufacturer's instructions. Prehybridization and hybridization solutions were prepared as previously described (Schmidt et al., Science 238:960-963 (1987), which is incorporated herein by reference). The probe for *ZAP1* RNA expression studies was a 445 bp SacI-NsiI fragment from the 3' end of the cDNA. Southern blot analyses were conducted to establish conditions for specific hybridization of this probe. No cross-hybridization was detected with

hybridization at 60°C in 50% formamide and washes at 65°C in 0.1x SSC and 0.5% SDS.

The strong sequence similarity between *ZAP1* and *AP1* indicated that *ZAP1* was the ortholog of this *Arabidopsis* floral meristem identity gene. As a first approximation of whether the pattern of *ZAP1* expression paralleled that of AP1, a blot of total RNA from vegetative and reproductive organs was hybridized with a gene-specific fragment of the *ZAP1* cDNA (nucleotides 370 to 820 of SEQ ID NO: 7). *ZAP1* RNA was detected only in male and female inflorescences and in the husk leaves that surround the developing ear. No *ZAP1* RNA expression was detectable in RNA isolated from root, shoot, leaf, endosperm, or embryo tissue. The restriction of *ZAP1* expression to terminal and axillary inflorescences is consistent with *ZAP1* being the *Arabidopsis AP1* ortholog.

Male and female florets were isolated from mature inflorescences, and the reproductive organs were separated from the remainder of the floret. RNA was isolated from the reproductive and the sterile portions of the florets. *ZAP1* RNA expression was not detected in maize stamens or carpels, whereas high levels of *ZAP1* RNA were present in developing ear and tassel florets from which the stamens and carpels had been removed. Thus, the exclusion of *ZAP1* expression in stamens and carpels and its inclusion in the RNA of the non-reproductive portions of the floret (lodicules, lemma and palea) is similar to the pattern of expression of AP1 in flowers of *Arabidopsis*.

EXAMPLE II

Conversion of shoot meristem to floral meristem in an APETALA1 transgenic plant

This example describes methods for producing a transgenic *Arabidopsis* plant, in which shoot meristem is converted to floral meristem.

A. Ectopic expression of APETALA1 converts inflorescence shoots into flowers

Transgenic plants that constitutively express *AP1* from the cauliflower mosaic virus 35S (CaMV35S) promoter were produced to determine whether ectopic *AP1* expression could convert shoot meristem to floral meristem. The *AP1* coding sequence was placed under control of the cauliflower mosaic virus 35S promoter (Odell et al., *supra*, 1985) as follows. BamHI linkers were ligated to the HincII site of the full-length *AP1* complementary DNA (Mandel et al., *supra*, (1992), which is incorporated herein by reference) in pAM116, and the resulting BamHI fragment was fused to the cauliflower mosaic virus 35S promoter (Jack et al., Cell 76:703-716 (1994), which is incorporated herein by reference) in pCGN18 to create pAM563.

Transgenic *AP1 Arabidopsis* plants of the Columbia ecotype were generated by selecting kanamycin-resistant plants after *Agrobacterium*-mediated plant transformation using the *in planta* method (Bechtold

et al., C.R. Acad. Sci. Paris 316:1194-1199 (1993), which is incorporated herein by reference). All analyses were performed in subsequent generations. Approximately 120 independent transgenic lines that displayed the described phenotypes were obtained.

Remarkably, in 35S-*AP1* transgenic plants, the normally indeterminate shoot apex) prematurely terminated as a floral meristem and formed a terminal flower. In addition, all lateral meristems that normally would produce inflorescence shoots also were converted into solitary flowers. These results demonstrate that ectopic expression of AP1 in shoot meristem is sufficient to convert shoot meristem to floral meristem, even though AP1 normally is not absolutely required to specify floral meristem identity.

B. *LEAFY* is not required for the conversion of inflorescence shoots to flowers in an *APETALA1* transgenic plant

To determine whether the 35S-*AP1* transgene causes ectopic *LFY* activity, and whether ectopic *LFY* activity is required for the conversion of shoot meristem to floral meristem, the 35S-*AP1* transgene was introduced into *Arabidopsis lfy* mutants. The 35S-*AP1* transgene was crossed into the strong *lfy-6* mutant background and the F_2 progeny were analyzed.

Lfy mutant plants containing the 35S-*AP1* transgene displayed the same conversion of apical and lateral shoot meristem to floral meristem as was observed in transgenics containing wild type LFY. However, the resulting flowers had the typical *lfy* mutant phenotype, in which floral organs developed as sepaloid and carpelloid structures, with an absence of petals and stamens. These results demonstrate that LFY is not required for the conversion of shoot meristem to floral meristem in a transgenic angiosperm that ectopically expresses AP1.

C. *APETALA1* is not sufficient to specify organ fate

As well as being involved in the early step of specifying floral meristem identity, *AP1* also is involved in specifying sepal and petal identity at a later stage in flower development. Although *AP1* RNA is initially expressed throughout the young flower primordium, it is later excluded from stamen and carpel primordia (Mandel et al., Nature 360:273-277 (1992)). Since the cauliflower mosaic virus 35S promoter is active in all floral organs, 35S-*AP1* transgenic plants are likely to ectopically express AP1 in stamens and carpels. However, 35S-*AP1* transgenic plants had normal stamens and carpels, indicating that AP1 is not sufficient to specify sepal and petal organ fate.

D. Ectopic expression of *APETALA1* causes early flowering

In addition to its ability to alter inflorescence meristem identity, ectopic expression of *AP1* also influences the vegetative phase of plant growth. Wild-type plants have a vegetative phase during which a basal rosette of leaves is produced, followed by the transition to reproductive growth. The transition from vegetative to reproductive growth was measured both in terms of the number of days post-germination until the first visible flowers were observed, and by counting the number of leaves. Under continuous light, wild-type and 35S-*AP1* transgenic plants flowered after producing 9.88 ± 1.45 and 4.16±0.97 leaves, respectively. Under short-day growth conditions (8 hours light, 16 hours dark, 24 C), wild-type and 35S-*AP1* transgenic plants flowered after producing 52.42±3.47 and 7.4±1.18 leaves, respectively.

In summary, under continuous light growth conditions, flowers appear on wild-type *Arabidopsis* plants after approximately 18 days, whereas the 35S-AP1 transgenic plants flowered after an average of only 10 days. Furthermore, under short-day growth conditions, flowering is delayed in wild-type plants until approximately 10 weeks after germination, whereas, 35S-*AP1* transgenic plants flowered in less than 3 weeks. Thus, ectopic *AP1* activity significantly reduced the time to flowering and reduced the delay of flowering caused by short day growth conditions.

EXAMPLE III

Isolation and characterization of the *Arabidopsis* and *Brassica oleracea CAULIFLOWER* genes

This example describes methods for isolating and characterizing the *Arabidopsis* and *Brassica oleracea CAL* genes.

A. Isolation of the *Arabidopsis* and *Brassica oleracea CAULIFLOWER* genes

Genetic evidence that CAL and AP1 proteins may be functionally related indicated that these proteins may share similar DNA sequences. In addition, DNA blot hybridization revealed that the *Arabidopsis* genome contains a gene that is closely related to *AP1*. The CAL gene, which is closely related to *AP1*, was isolated and identified as a member of the family of *Arabidopsis* MADS domain genes known as the *AGAMOUS*-like (*AGL*) genes.

Hybridization with an *AP1* probe was used to isolate a 4.8-kb Eco RI genomic fragment of *CAL*. The corresponding CAL complementary DNA (pBS85) was cloned by reverse transcription-polymerase chain reaction (RT-PCR) with the oligonucleotides AGL10-1 (5'-GATCGTCGTTATCTCTCTTG-3'; SEQ ID NO: 25) and AGL10-12 (5'-GTAGTCTATTCAAGCGGCG-3'; SEQ ID NO: 26).

The *Arabidopsis CAL* cDNA encodes a putative 255 amino acid protein (Figure 5; SEQ ID NO: 10) having a calculated molecular weight of 30.1 kD and an isoelectric

point of 8.78. The deduced amino acid sequence for *CAL* contains a MADS domain which generally is present in a class of transcription factors. The MADS domains of CAL and AP1 were markedly similar, differing in only 5 of 56 amino acid residues, 4 of which represent conservative replacements. Overall, the putative CAL protein is 76% identical to AP1; with allowance for conservative amino acid substitutions, the two proteins are 88% similar. These results indicate that CAL and AP1 may recognize similar target sequences and regulate many of the same genes involved in floral meristems identity.

CAL was mapped to the approximate location of the loci identified by classical genetic means for the cauliflower phenotype (Bowman et al., Development 119:721 (1993), which is herein incorporated by reference). Restriction fragment length polymorphism (RFLP) mapping filters were scored and the results analyzed with the Macintosh version of the Mapmaker program as described by Rieter et al., (Proc. Natl. Acad. Sci., USA, 89:1477 (1992), which is herein incorporated by reference). The results localized *CAL* to the upper arm of chromosome 1, near marker λ235.

A genomic fragment spanning the CAL gene was used to transform *cal-1 ap1-1* plants. A 5850-bp Bam HI fragment containing the entire coding region of the *Arabidopsis CAL* gene as well as 1860 bp upstream of the putative translational start site was inserted into the pBIN19 plant transformation vector (Clontech, Palo Alto, California) and used for transformation of root tissue

from *cal-1 ap1-1* plants as described by Valvekens et al. (Proc. Natl. Acad. Sci., USA 85:5536 (1988), which is incorporated herein by reference). Seeds were harvested from primary transformants, and all phenotypic analyses were performed in subsequent generations. Four independent lines transformed with CAL showed a complementation of the *cauliflower (cal)* phenotype and displayed a range of phenotypes similar to those exhibited by *ap1* mutants. These results demonstrated that CAL functions to convert shoot meristem to floral meristem.

In order to identify regions of functional importance in the CAL protein, *cal* mutants were generated and analyzed. The *cal* alleles were isolated by mutagenizing seeds homozygous for the *ap1-1* allele in Ler with 0.1% or 0.05% ethylmethane sulfonate (EMS) for 16 hours. Putative new *cal* alleles were crossed to *cal-1 ap1-1 chlorina* plants to verify allelism. Two sets of oligonucleotides were used to amplify and clone new alleles: oligos AGL10-1 (SEQ ID NO: 25) and AGL10-2 (5'-GATGGAGACCATTAAACAT-3; SEQ ID NO: 27) for the 5' portion and oligos AGL10-3 (5'-GGAGAAGGTACTAGAACG-3'; SEQ ID NO: 28) and AGL10-4 (5'-GCCCTCTTCCATAGATCC-3'; SEQ ID NO: 29) for the 3' portion of the gene. All coding regions and intron-exon boundaries of the mutant alleles were sequenced.

Sequence analysis of the *cal-1* allele, which exists in the wild-type Wassilewskija (WS) ectoype, revealed a cluster of three amino acid differences in the

seventh exon, relative to th wild-type gene product from Landsberg *erecta* (Ler) (Figure 8). One or more of these amino acid differences can be responsible for the *cal* phenotype, because the *cal-1* gene was expressed normally and the transcribed RNA was correctly spliced in the WS background. The three additional *cal* alleles that were isolated, designated *cal-2*, *cal-3*, and *cal-4*, exhibited phenotypes similar to that of the *cal-1* allele.

Sequence analyses revealed a single missense mutation for each (Figure 8). Since mutations in the *cal-2* and *cal-3* alleles lie in the MADS domain, these mutations can affect the ability of CAL to bind DNA and activate its target genes. Because the *cal-4* allele contains a substitution in the K domain, a motif thought to be involved in protein-protein interactions, this mutation can affect the ability of CAL to form homodimers or to interact with other proteins such as AP1.

B. RNA expression pattern of CAULIFLOWER

To characterize the temporal and spatial pattern of *CAL* RNA accumulation, RNA *in situ* hybridizations were performed using a CAL-specific probe. ^{35}S-labeled antisense *CAL* and *BoCAL* mRNA was synthesized from Sca 1-digested cDNA templates and hybridized to 8 μm sections of *Arabidopsis* Ler or *Brassica oleracea* inflorescences. The probes did not contain any MADS box sequences in order to avoid cross-hybridization with other MADS box genes. Hybridization conditions were as

previously described (Drews et al., Cell 65:991 (1991), which is herein incorporated by reference).

As with *AP1*, *CAL* RNA accumulated in young flower primordia, consistent with the ability of CAL to substitute for AP1 in specifying floral meristems. In contrast to *AP1* RNA, however, which accumulated at high levels throughout sepal and petal development, *CAL* RNA was detected only at very low levels in these organs. These results demonstrate that *CAL* was unable to substitute for *AP1* in specifying sepals and petals, at least in part as a result of the relatively low levels of *CAL* RNA in these developing organs.

C. Molecular Basis of the *cauliflower* phenotype

The *cal* phenotype in *Arabidopsis* is similar to the inflorescence structure that develops in the closely related species *Brassica oleracea* var. *botrytis*, the cultivated garden variety of cauliflower, indicating that the *CAL* gene can contribute to the *cal* phenotype of this agriculturally important species. Thus, *CAL* gene homologs were isolated from a *Brassica oleracea* line that produces wild-type flowers (*BoCAL*) and from the common garden variety of cauliflower *Brassica oleracea* var. *botrytis* (*BobCAL*).

The single-copy *BobCAL* gene (Snowball Y Improved, NK Lawn & Garden, Minneapolis, MN) was isolated from a size-selected genomic library in λBlueStar (Novagen) on a 16-kbp BamHI fragment with the *Arabidopsis*

CAL gene as a probe. The *BoCAL* gene was isolated from a rapid cycling line (Williams and Hill, Science 232:1385 (1986)) by PCR on both RNA and genomic DNA. The cDNA was isolated by RT-PCR using the oligonucleotides: Bob1 (5'-TCTACGAGAAATGGGAAGG-3'; SEQ ID NO: 30) and Bob2 (5'-GTCGATATATGGCGAGTCC-3'; SEQ ID NO: 31). The 5' portion of the gene was obtained using oligonucleotides Bob 1 (SEQ ID NO: 30) and Bob4B (5'-CCATTGACCAGTTCGTTTG-3'; SEQ ID NO: 32). The 3' portion was obtained using oligonucleotides Bob3 (5'-GCTCCAGACTCTCACGTC-3'; SEQ ID NO: 33) and Bob2 (SEQ ID NO: 31).

RNA *in situ* hybridizations were performed to determine the expression pattern of *BoCAL* gene from *Brassica oleracea*. As in *Arabidopsis*, *BoCAL* RNA accumulated uniformly in early floral primordia and later was excluded from the cells that give rise to stamens and carpels.

DNA sequence analyses revealed that the open reading frame of the *BoCAL* gene is intact, whereas that of the *BobCAL* gene is interrupted by a stop codon in exon 5 (Figure 8). Translation of the resulting BobCAL protein product is truncated after only 150 of the wild-type 255 amino acids. Because similar stop codon mutations in the fifth exon of the *Arabidopsis* AP1 coding sequence result in plants having a severe *ap1* phenotype, the BobCAL protein likely is not functional. These results indicate that, as in *Arabidopsis*, the molecular basis for the *cauliflower* phenotype in *Brassica oleracea*

var. *botrytis* is due, at least in part, to a mutation in the *BobCAL* gene.

EXAMPLE IV

Conversion of inflorescence shoots into flowers in an CAULIFLOWER transgenic plant

This example describes methods for producing a transgenic CAL plant.

A. Ectopic expression of *CAULIFLOWER* converts inflorescence shoots to flowers

Transgenic *Arabidopsis* plants that ectopically express *CAL* in shoot meristem were generated. The full-length *CAL* cDNA was inserted downstream of the 35S cauliflower mosaic virus promoter in the EcoRI of pMON530 (Monsanto Co. Co., St. Louis, Missouri) This plasmid was introduced into Agrobacterium strain ASE (check) and used to transform the Columbia ecotype of *Arabidopsis* using a modified vacuum infiltration method described by Bechtold et al. (*supra*, 1993). The 96 lines generated that harbored the 35S-*CAL* construct had a range of weak to strong phenotypes. The transgenic plants with the strongest phenotypes (27 lines) closely resembled the *tfl* mutant.

35S-*CAL* transgenic plants had converted apical and lateral inflorescence shoots into flowers and showed an early flowering phenotype. These results demonstrate

that *CAL* is sufficient for the conversion of shoots to flowers and for promoting early flowering.

EXAMPLE V

Conversion of shoots into flowers in a LEAFY transgenic plant

This example describes methods for producing a transgenic *LFY Arabidopsis* and aspen.

A. Conversion of *Arabidopsis* shoots by *LEAFY*

Transgenic *Arabidopsis* plants were generated by transforming *Arabidopsis* with *LFY* under the control of the cauliflower mosaic virus 35S promoter (CaMV35S)(Odell et al., *supra*, (1985)). A *LFY* complementary cDNA (Weigel et al, Cell 69:843-859 (1992), which is incorporated herein by reference) was inserted into a T-DNA transformation vector containing a CaMV *35S* promoter/3' *nos* cassette (Jack et al., *supra*, 1994). Transformed seedlings were selected for kanamycin resistance. Several hundred transformants in three different genetic backgrounds (Nossen, Wassilewskija and Columbia) were recovered and several lines were characterized in detail.

High levels of *LFY* RNA expression were detected by northern blot analysis. In general, Nossen lines had weaker phenotypes, especially when grown in short days. The *35S-LFY* transgene of line *DW151.117* (ecotype Wassilewskija) was introgressed into the *erecta* background by backcrossing to a Landsberg *erecta* strain.

Plants were grown under 16 hours light and 8 hours dark. The *35S-LFY* transgene provided at least as much *LFY* activity as the endogenous gene and completely suppressed the *lfy* mutant phenotype when crossed into the background of the *lfy-6* null allele.

Most *35S-LFY* transgenic plants lines demonstrated a very similar, dominant and heritable phenotype. Secondary shoots that arose in lateral positions were consistently replaced by solitary flowers, and higher-order shoots were absent. Although the number of rosette leaves was unchanged from the wild type, *35S-LFY* plants flowered earlier than wild type; the solitary flowers in the axils of the rosette leaves developed and opened precociously. In addition, the primary shoot terminated with a flower. In the most extreme cases, a terminal flower was formed immediately above the rosette. This gain of function phenotype (conversion of shoots to flowers) is the opposite of the *lfy* loss of function phenotype (conversion of flowers to shoots). These results demonstrate that *LFY* encodes a developmental switch that is both sufficient and necessary to convert shoot meristem to flower meristem.

The effects of constitutive *LFY* expression differ for primary and secondary shoot meristems. Secondary meristems were transformed into flower meristem, apparently as soon as it developed, and produced only a single, solitary flower. In contrast, primary shoot meristem produced leaves and lateral flowers before being consumed in the formation of a

terminal flower. These developmental differences indicate that a meristem must acquire competence to respond to the activity of a floral meristem identity gene such as *LFY*.

B. Conversion of aspen shoots by *LEAFY*

Given that constitutive expression of *LFY* induced precocious flowering during the vegetative phase of *Arabidopsis*, the effect of *LFY* on the flowering of other species was examined. The perennial tree, hybrid aspen, is derived from parental species that flower naturally only after 8-20 years of growth (Schopmeyer (ed.), USDA Agriculture Handbook 450: Seeds of Woody Plants in the United States, Washington DC, USA: US Government Printing Office, pp. 645-655 (1974)). *35S-LFY* aspen plants were obtained by *Agrobacterium*-mediated transformation of stem segments and subsequent regeneration of transgenic shoots in tissue culture.

Hybrid aspen was transformed exactly as described by Nilsson et al. (Transgen. Res. 1:209-220 (1992), which is incorporated herein by reference). Levels of *LFY* RNA expression were similar to those of *35S-LFY Arabidopsis*, as determined by northern blot analysis. The number of vegetative leaves varied between different regenerating shoots, and those with a higher number of vegetative leaves formed roots, allowing for transfer to the greenhouse. Individual flowers were removed either from primary transformants that had been transferred to the greenhouse, or from catkins collected

in spring, 1995, at Carlshem, Umeå, Sweden) from a tree whose age was determined by counting the number of annual rings in a core extracted with an increment borer at 1.5 meters above ground level. Flowers were fixed in formaldehyde/acetic acid/ethanol and destained in ethanol before photography.

The overall phenotype of *35S-LFY* aspen was similar to that of *35S-LFY Arabidopsis*. In wild-type plants of both species, flowers normally are formed in lateral positions on inflorescence shoots. In aspen, these inflorescence shoots, called catkins, arise from the leaf axils of adult trees. In both *35S-LFY Arabidopsis* and *35S-LFY* aspen, solitary flowers were formed instead of shoots in the axils of vegetative leaves. Moreover, as in *Arabidopsis*, the secondary shoots of trangenic aspen were more severely affected than the primary shoot.

Regenerating *35S-LFY* aspen shoots initially produced solitary flowers in the axils of normal leaves. However, the number of vegetative leaves was limited, and the shoot meristem was prematurely consumed in the formation of an aberrant terminal flower. Precocious flower development was specific to *35S-LFY* transformants and was not observed in non-transgenic controls. Furthermore, not a single instance of precocious flower development has been observed in more than 1,500 other lines of transgenic aspen generated with various constructs from 1989 to 1995 at the Swedish University of Agricultural Sciences. These results demonstrate that a

heterologous floral meristem identity gene product is active in an angiosperm.

Although the invention has been described with reference to the examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

We claim:

1. A nucleic acid molecule encoding a CAULIFLOWER (CAL) gene product having at least about 70 percent amino acid identity with amino acids 1 to 160 of the sequence shown in Figure 5 (SEQ ID NO: 10) or with amino acids 1 to 160 of the sequence shown in Figure 6 (SEQ ID NO: 12).

2. The nucleic acid molecule of claim 1, wherein said CAL gene product is selected from the group consisting of *Arabidopsis thaliana* CAL having the amino acid sequence shown in Figure 5 (SEQ ID NO: 10) and *Brassica oleracea* CAL having the amino acid sequence shown in Figure 6 (SEQ ID NO: 12).

3. A nucleic acid molecule selected from the group consisting of a nucleic acid molecule having the nucleic acid sequence shown in Figure 5 (SEQ ID NO: 9) and a nucleic acid molecule having the nucleic acid sequence shown in Figure 6 (SEQ ID NO: 11).

4. A nucleic acid molecule encoding a truncated CAL gene product having at least about 70 percent amino acid identity with amino acids 1 to 150 of the sequence shown in Figure 7 (SEQ ID NO: 14).

5. The nucleic acid molecule of claim 4, wherein said truncated CAL gene product is *Brassica oleracea* var. *botrytis* CAL having the amino acid sequence shown in Figure 7 (SEQ ID NO: 14).

6. A nucleic acid molecule having the nucleic acid sequence shown in Figure 7 (SEQ ID NO: 13).

7. A nucleotide sequence that hybridizes under relatively stringent conditions to a nucleic acid molecule selected from the group consisting of:

the nucleic acid molecule of claim 3 or a nucleic acid molecule complementary thereto; and

the nucleic acid molecule of claim 6 or a nucleic acid molecule complementary thereto.

8. A *CAL* gene, comprising a *CAL* gene selected from the group consisting of an *Arabidopsis thaliana CAL* gene having the nucleotide sequence shown in Figure 13 (SEQ ID NO: 20), a *Brassica oleracea CAL* gene having the nucleotide sequence shown in Figure 14 (SEQ ID NO: 21) and a *Brassica oleracea* var. *botrytis CAL* gene having the nucleotide sequence shown in Figure 15 (SEQ ID NO: 22).

9. A nucleotide sequence that hybridizes under relatively stringent conditions to the *CAL* gene of claim 8, or a complementary sequence thereto.

10. A vector, comprising the nucleic acid molecule of claim 1.

11. A vector, comprising the gene of claim 8.

12. A vector, comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecule of claim 2 and the nucleic acid molecule of claim 3.

13. A host cell, comprising the vector of claim 10.

14. Th vector of claim 10, wherein said vector is an expression vector.

15. An expression vector, comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecule of claim 2 and the nucleic acid molecule of claim 3.

16. The expression vector of claim 14, further comprising a cauliflower mosaic virus 35S promoter.

17. The expression vector of claim 14, further comprising an inducible regulatory element.

18. A kit for converting shoot meristem to floral meristem in an angiosperm, comprising the expression vector of claim 14.

19. A kit for promoting early flowering in an angiosperm, comprising the expression vector of claim 14.

20. A CAL polypeptide having at least about 70 percent amino acid identity with amino acids 1 to 160 of the sequence shown in Figure 5 (SEQ ID NO: 10) or with amino acids 1 to 160 of the sequence shown in Figure 6 (SEQ ID NO: 12).

21. The CAL polypeptide of claim 20, wherein said CAL polypeptide is *Arabidopsis thaliana* CAL polypeptide having the amino acid sequence shown as amino acids 1 to 255 in Figure 5 (SEQ ID NO: 10).

22. The CAL polypeptide of claim 20, wherein said CAL polypeptide is *Brassica oleracea* CAL polypeptide having the amino acid sequence shown as amino acids 1 to 255 in Figure 6 (SEQ ID NO: 12).

23. An antibody that specifically binds the CAL polypeptide of claim 20.

24. The antibody of claim 23, wherein said antibody is a monoclonal antibody.

25. A truncated *Brassica oleracea* var. *botrytis* CAL polypeptide having the amino acid sequence shown as amino acids 1 to 150 in Figure 7 (SEQ ID NO: 14).

26. An antibody that specifically binds the truncated *Brassica oleracea* var. *botrytis* CAL polypeptide of claim 25.

27. A method of identifying a *Brassica* having a modified *CAL* allele, comprising detecting a polymorphism associated with a *CAL* locus, said *CAL* locus comprising a modified *CAL* allele that does not encode an active CAL gene product.

28. The method of claim 27, wherein said modified *CAL* allele encodes a truncated CAL gene product.

29. The method of claim 27, wherein said polymorphism is within a *CAL* gene.

30. The method of claim 29, wherein said polymorphism is detectable as a restriction fragment length polymorphism.

31. The method of claim 30, wherein said polymorphism is at nucleotide 451 of the nucleic acid sequence shown in Figure 7 (SEQ ID NO: 13).

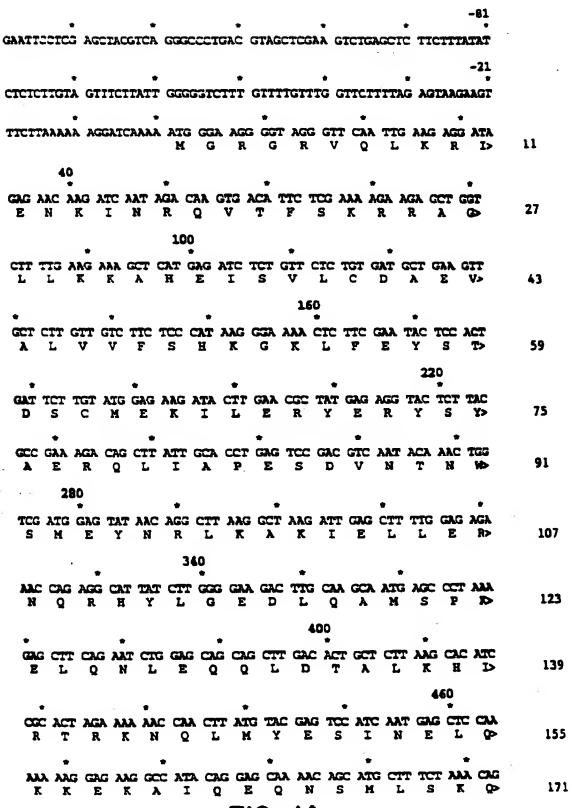
-81
GAATTCCTCG AGCTACGTCA GGGCCCTGAC GTAGCTCGAA GTCTGAGCTC TTCTTTATAT
-21
CTCTCTTGTA GTTTCTTATT GGGGGTCTTT GTTTTGTTTG GTTCTTTTAG AGTAAGAAGT
TTCTTAAAAA AGGATCAAAA ATG GGA AGG GGT AGG GTT CAA TTG AAG AGG ATA
M G R G R V Q L K R I 11
40
GAG AAC AAG ATC AAT AGA CAA GTG ACA TTC TCG AAA AGA AGA GCT GGT
E N K I N R Q V T F S K R R A G 27
100
CTT TTG AAG AAA GCT CAT GAG ATC TCT GTT CTC TGT GAT GCT GAA GTT
L L K K A H E I S V L C D A E V 43
160
GCT CTT GTT GTC TTC TCC CAT AAG GGA AAA CTC TTC GAA TAC TCC ACT
A L V V F S H K G K L F E Y S T 59
220
GAT TCT TGT ATG GAG AAG ATA CTT GAA CGC TAT GAG AGG TAC TCT TAC
D S C M E K I L E R Y E R Y S Y 75
GCC GAA AGA CAG CTT ATT GCA CCT GAG TCC GAC GTC AAT ACA AAC TGG
A E R Q L I A P E S D V N T N W 91
280
TCG ATG GAG TAT AAC AGG CTT AAG GCT AAG ATT GAG CTT TTG GAG AGA
S M E Y N R L K A K I E L L E R 107
340
AAC CAG AGG CAT TAT CTT GGG GAA GAC TTG CAA GCA ATG AGC CCT AAA
N Q R H Y L G E D L Q A M S P K 123
400
GAG CTT CAG AAT CTG GAG CAG CAG CTT GAC ACT GCT CTT AAG CAC ATC
E L Q N L E Q Q L D T A L K H I 139
460
CGC ACT AGA AAA AAC CAA CTT ATG TAC GAG TCC ATC AAT GAG CTC CAA
R T R K N Q L M Y E S I N E L Q 155
AAA AAG GAG AAG GCC ATA CAG GAG CAA AAC AGC ATG CTT TCT AAA CAG
K K E K A I Q E Q N S M L S K Q 171

FIG IA

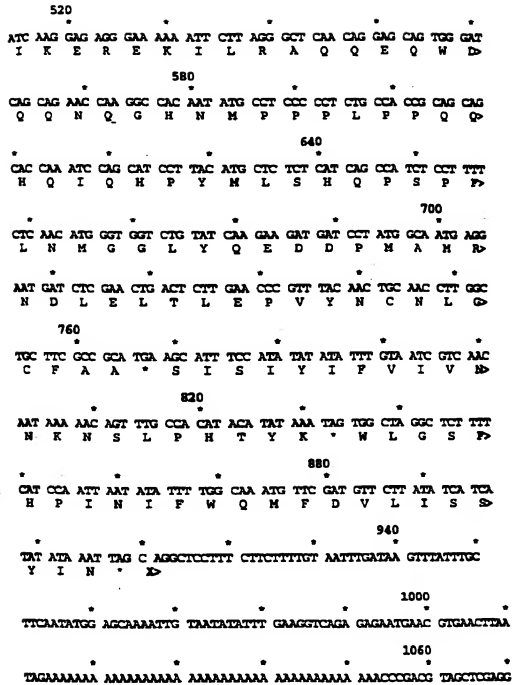
520
ATC AAG GAG AGG GAA AAA ATT CTT AGG GCT CAA CAG GAG CAG TGG GAT
I K E R E K I L R A Q Q E Q W D
187
580
CAG CAG AAC CAA GGC CAC AAT ATG CCT CCC CCT CTG CCA CCG CAG CAG
Q Q N Q G H N M P P P L P P Q Q
203
640
CAC CAA ATC CAG CAT CCT TAC ATG CTC TCT CAT CAG CCA TCT CCT TTT
H Q I Q H P Y M L S H Q P S P F
219
700
CTC AAC ATG GGT GGT CTG TAT CAA GAA GAT GAT CCT ATG GCA ATG AGG
L N M G G L Y Q E D D P M A M R
235
AAT GAT CTC GAA CTG ACT CTT GAA CCC GTT TAC AAC TGC AAC CTT GGC
N D L E L T L E P V Y N C N L G
251
760
TGC TTC GCC GCA TGA AGC ATT TCC ATA TAT ATA TTT GTA ATC GTC AAC
C F A A * S I S I Y I F V I V N
267
820
AAT AAA AAC AGT TTG CCA CAT ACA TAT AAA TAG TGG CTA GGC TCT TTT
N K N S L P H T Y K * W L G S F
283
880
CAT CCA ATT AAT ATA TTT TGG CAA ATG TTC GAT GTT CTT ATA TCA TCA
H P I N I F W Q M F D V L I S S
299
940
TAT ATA AAT TAG C AGGCTCCTTT CTTCTTTTGT AATTTGATAA GTTTATTTGC
Y I N * R
302
1000
TTCAATATGG AGCAAAATTG TAATATATTT GAAGGTCAGA GAGAATGAAC GTGAACTTAA
1060
TAGAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAACCCGACG TAGCTCGAGG
AATTC

FIG. IB

```
                                                                    .                   .
TCTTAGAGGA AATAGTTCCT TTAAAAGGGA TAAAA ATG GGA AGG GGT AGG GTT CAG                          7
                                       M   G   R   G   R   V   Q
  25
  .                 .                 .                 .                 .
TTG AAG AGG ATA GAA AAC AAG ATC AAT AGA CAA GTG ACA TTC TCG AAA                            23
L   K   R   I   E   N   K   I   N   R   Q   V   T   F   S   K
                  .85               .                 .                 .
AGA AGA GCT GGT CTT ATG AAG AAA GCT CAT GAG ATC TCT GTT CTG TGT                            39
R   R   A   G   L   M   K   K   A   H   E   I   S   V   L   C
                  .                 .145              .                 .
GAT GCT GAA GTT GCG CTT GTT GTC TTC TCC CAT AAG GGG AAA CTC TTT                            55
D   A   E   V   A   L   V   V   F   S   H   K   G   K   L   F
                  .                 .                 .205              .
GAA TAC TCC ACT GAT TCT TGT ATG GAG AAG ATA CTT GAA CGC TAT GAG                            71
E   Y   S   T   D   S   C   M   E   K   I   L   E   R   Y   E
                  .                 .                 .                 .
AGA TAC TCT TAC GCC GAG AGA CAG CTT ATA GCA CCT GAG TCC GAC TCC                            87
R   Y   S   Y   A   E   R   Q   L   I   A   P   E   S   D   S
  265
  .                 .                 .                 .                 .
AAT ACG AAC TGG TCG ATG GAG TAT AAT AGG CTT AAG GCT AAG ATT GAG                            103
N   T   N   W   S   M   E   Y   N   R   L   K   A   K   I   E
                  .325              .                 .                 .
CTT TTG GAG AGA AAC CAG AGG CAC TAT CTT GGG GAA GAC TTG CAA GCA                            119
L   L   E   R   N   Q   R   H   Y   L   G   E   D   L   Q   A
                  .                 .385              .                 .
ATG AGC CCT AAG GAA CTC CAG AAT CTA GAG CAA CAG CTT GAT ACT GCT                            135
M   S   P   K   E   L   Q   N   L   E   Q   Q   L   D   T   A
                  .                 .                 .445              .
CTT AAG CAC ATC CGC TCT AGA AAA AAC CAA CTT AGT TAC GAC TCC ATC                            151
L   K   H   I   R   S   R   K   N   Q   L   M   Y   D   S   I
                  .                 .                 .                 .
AAT GAG CTC CAA AGA AAG GAG AAA GCC ATA CAG GAA CAA AAC AGC ATG                            167
N   E   L   Q   R   K   E   K   A   I   Q   E   Q   N   S   M
  505
  .                 .                 .                 .                 .
CTT TCC AAG CAG ATT AAG GAG AGG GAA AAC GTT CTT AGG GCG CAA CAA                            183
L   S   K   Q   I   K   E   R   E   N   V   L   R   A   Q   Q
```

FIG. 2A

```
                565
GAG CAA TGG GAC GAG CAG AAC CAT GGC CAT AAT ATG CCT CCG CCT CCA
 E   Q   W   D   E   Q   N   H   G   H   N   M   P   P   P   P    199

                                625
CCC CCG CAG CAG CAT CAA ATC CAG CAT CCT TAC ATG CTC TCT CAT CAG
 P   P   Q   Q   H   Q   I   Q   H   P   Y   M   L   S   H   Q    215

                                            685
CCA TCT CCT TTT CTC AAC ATG GGG GGG CTG TAT CAA GAA GAA GAT CAA
 P   S   P   F   L   N   M   G   G   L   Y   Q   E   E   D   Q    231

ATG GCA ATG AGG AGG AAC GAT CTC GAT CTG TCT CTT GAA CCC GGT TAT
 M   A   M   R   R   N   D   L   D   L   S   L   E   P   G   Y    247

 745
AAC TGC AAT CTC GGC TGC
 N   C   N   L   G   C                                            253
```

FIG. 2B

```
ATG GGA AGG GGT AGG GTT CAG TTG AAG AGG ATA GAA AAC AAG ATC AAT
 M   G   R   G   R   V   Q   L   K   R   I   E   N   K   I   N      16

          60
AGA CAA GTG ACA TTC TCG AAA AGA AGA GCT GGT CTT ATG AAG AAA GCT
 R   Q   V   T   F   S   K   R   R   A   G   L   M   K   K   A      32

                  120
CAT GAG ATC TCT GTT CTG TGT GAT GCT GAA GTT GCG CTT GTT GTC TTC
 H   E   I   S   V   L   C   D   A   E   V   A   L   V   V   F      48

                          180
TCC CAT AAG GGG AAA CTC TTT GAA TAC CCC ACT GAT TCT TGT ATG GAG
 S   H   K   G   K   L   F   E   Y   P   T   D   S   C   M   E      64

                                  240
GAG ATA CTT GAA CGC TAT GAG AGA TAC TCT TAC GCC GAG AGA CAG CTT
 E   I   L   E   R   Y   E   R   Y   S   Y   A   E   R   Q   L      80

ATA GCA CCT GAG TCC GAC TCC AAT ACG AAC TGG TCG ATG GAG TAT AAT
 I   A   P   E   S   D   S   N   T   N   W   S   M   E   Y   N      96

          300
AGG CTT AAG GCT AAG ATT GAG CTT TTG GAG AGA AAC CAG AGG CAC TAT
 R   L   K   A   K   I   E   L   L   E   R   N   Q   R   H   Y     112

                  360
CTT GGG GAA GAC TTG CAA GCA ATG AGC CCT AAG GAA CTC CAG AAT CTA
 L   G   E   D   L   Q   A   M   S   P   K   E   L   Q   N   L     128

                          420
GAG CAA CAG CTT GAT ACT GCT CTT AAG CAC ATC CGC TCT AGA AAA AAC
 E   Q   Q   L   D   T   A   L   K   H   I   R   S   R   K   N     144

                                  480
CAA CTT ATG TAC GAC TCC ATC AAT GAG CTC CAA AGA AAG GAG AAA GCC
 Q   L   M   Y   D   S   I   N   E   L   Q   R   K   E   K   A     160

ATA CAG GAA CAA AAC AGC ATG CTT TCC AAG CAG ATT AAG GAG AGG GAA
 I   Q   E   Q   N   S   M   L   S   K   Q   I   K   E   R   E     176

          540
AAC GTT CTT AGG GCG CAA CAA GAG CAA TGG GAC GAG CAG AAC CAT GGC
 N   V   L   R   A   Q   Q   E   Q   W   D   E   Q   N   H   G     192
```

FIG. 3A

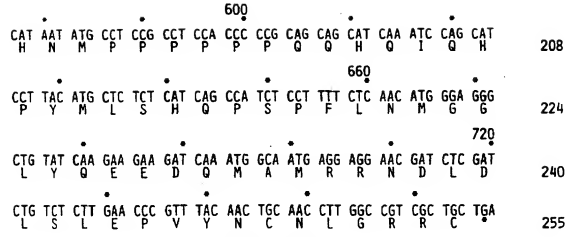
600
CAT AAT ATG CCT CCG CCT CCA CCC CCG CAG CAG CAT CAA ATC CAG CAT
H N M P P P P P P Q Q H Q I Q H
208
660
CCT TAC ATG CTC TCT CAT CAG CCA TCT CCT TTT CTC AAC ATG GGA GGG
P Y M L S H Q P S P F L N M G G
224
720
CTG TAT CAA GAA GAA GAT CAA ATG GCA ATG AGG AGG AAC GAT CTC GAT
L Y Q E E D Q M A M R R N D L D
240
CTG TCT CTT GAA CCC GTT TAC AAC TGC AAC CTT GGC CGT CGC TGC TGA
L S L E P V Y N C N L G R R C *
255

FIG. 3B

```
            GCACGAGTCCTCCTCCTCCTCGCATCCCACCCCACCCCACCTTCTCCTTAAAGCTACCTGCCTACCCGG
CGGTTGCGCGCCGCAATCGATCGACCGGAAGAGAAAGAGCAGCTAGCTAGCTAGCAGATCGGAGCACGGCAACAAGGCG

ATG GGG CGC GGC AAG GTA CAG CTG AAG CGG ATA GAG AAC AAG ATA AAC CGG CAG GTG ACC    60
 M   G   R   G   K   V   Q   L   K   R   I   E   N   K   I   N   R   Q   V   T     20

TTC TCC AAG CGC CGG AAC GGC CTG CTC AAG AAG GCG CAC GAG ATC TCC GTC CTC TGC GAT   120
 F   S   K   R   R   N   G   L   L   K   K   A   H   E   I   S   V   L   C   D     40

GCC GAG GTC GCC GTC ATC GTC TTC TCC CCC AAG GGC AAG CTC TAC GAG TAC GCC ACC GAC   180
 A   E   V   A   V   I   V   F   S   P   K   G   K   L   Y   E   Y   A   T   D     60

TCC CGC ATG GAC AAA ATT CTT GAA CGC TAT GAG CGA TAT TCC TAT GCT GAA AAG GCT CTT   240
 S   R   M   D   K   I   L   E   R   Y   E   R   Y   S   Y   A   E   K   A   L     80

ATT TCA GCT GAA TCT GAA AGT GAG GGA AAT TGG TGC CAC GAA TAC AGG AAA CTG AAG GCC   300
 I   S   A   E   S   E   S   E   G   N   W   C   H   E   Y   R   K   L   K   A    100

AAA ATT GAG ACC ATA CAA AAA TGC CAC AAG CAC CTG ATG GGA GAG GAT CTA GAG TCT TTG   360
 K   I   E   T   I   Q   K   C   H   K   H   L   M   G   E   D   L   E   S   L    120

AAT CCC AAA GAG CTC CAG CAA CTA GAG CAG CAG CTG GAT AGC TCA CTG AAG CAC ATC AGA   420
 N   P   K   E   L   Q   Q   L   E   Q   Q   L   D   S   S   L   K   H   I   R    140

TCA AGG AAG AGC CAC CTT ATG GCC GAG TCT ATT TCT GAG CTA CAG AAG AAG GAG AGG TCA   480
 S   R   K   S   H   L   M   A   E   S   I   S   E   L   Q   K   K   E   R   S    160

CTG CAG GAG GAG AAC AAG GCT CTG CAG AAG GAA CTT GCG GAG AGG CAG AAG GCC GTC GCG   540
 L   Q   E   E   N   K   A   L   Q   K   E   L   A   E   R   Q   K   A   V   A    180

AGC CGG CAG CAG CAG CAA CAG CAG CAG GTG CAG TGG GAC CAG CAG ACA CAT GCC CAG GCC   600
 S   R   Q   Q   Q   Q   Q   Q   Q   V   Q   W   D   Q   Q   T   H   A   Q   A    200

CAG ACA AGC TCA TCA TCG TCC TCC TTC ATG ATG AGG CAG GAT CAG CAG GGA CTG CCG CCT   660
 Q   T   S   S   S   S   S   S   F   M   M   R   Q   D   Q   Q   G   L   P   P    220
```

FIG. 4A

```
CCA CAC AAC ATC TGC TTC CCG CCG TTG ACA ATG GGA GAT AGA GGT GAA GAG CTG GCT GCG   720
 P   H   N   I   C   F   P   P   L   T   M   G   D   R   G   E   E   L   A   A   240

GCG GCG GCG GCG CAG CAG CAG CAG CCA CTG CCG GGG CAG GCG CAA CCG CAG CTC CGC ATC   780
 A   A   A   A   Q   Q   Q   Q   P   L   P   G   Q   A   Q   P   Q   L   R   I   260

GCA GGT CTG CCA CCA TGG ATG CTG AGC CAC CTC AAT GCA TAA GGAGAGGGTCGATGAACACATCG   845
 A   G   L   P   P   W   M   L   S   H   L   N   A   *                         273

ACCTCCTCCTCTCTCTCTCGTCATGGATCATGACGTACGGCGTACCATATGGTTGCTGTGCCTGCCCCCATCGATCG   924
CGAGCAATGGCAACGCTCATGCAAGTGATCATTGCTCCCCGTTGGTTAAACCCTAGCCTATGTTCATGGCGTCAGCAACT  1003
AAGGTAAAACTATTGTTATGTTGCAAGAAAGGGTAAACCCGCTAGCTGTGTAATCTTGTCCAGCTATCAGTATGCTTGT  1082
TACTGCCCAGTTACCCTTGAATCTAGCGGGCGCTTTTGGTGAGAGGGTGCAGTTTACTTTAAACATGGTTCGTGACTTGC  1161
TGTAAATAGTAGTATTAAATCGATTTGGGCATCT(A)n                                          1195
```

FIG. 4B

```
TTAAAAGAA ATG GGA AGG GGT AGG GTT GAA TTG AAG AGG ATA GAG AAC AAG
          M  G  R  G  R  V  E  L  K  R  I  E  N  K>          14

      51
AIC AAT AGA CAA GTG ACA TTC TCG AAA AGA AGA ACT GGT CTT TTG AAG
 I  N  R  Q  V  T  F  S  K  R  R  T  G  L  L  K>          30

             111
AAA GCT CAG GAG ATC TCT GTT CTT TGT GAT GCC GAG GTT TCC CTT ATT
 K  A  Q  E  I  S  V  L  C  D  A  E  V  S  L  I>          46

                         171
GTC TTC TCC CAT AAG GGC AAA TTG TTC GAG TAC TCC TCT GAA TCT TGC
 V  F  S  H  K  G  K  L  F  E  Y  S  S  E  S  C>          62

                                     231
ATG GAG AAG GTA CTA GAA CGC TAC GAG AGG TAT TCT TAC GCC GAG AGA
 M  E  K  V  L  E  R  Y  E  R  Y  S  Y  A  E  R>          78

CAG CTG ATT GCA CCT GAC TCT CAC GTT AAT GCA CAG ACG AAC TGG TCA
 Q  L  I  A  P  D  S  H  V  N  A  Q  T  N  W  S>          94

      291
ATG GAG TAT AGC AGG CTT AAG GCC AAG ATT GAG CTT TTG GAG AGA AAC
 M  E  Y  S  R  L  K  A  K  I  E  L  L  E  R  N>         110

             351
CAA AGG CAT TAT CTG GGA GAA GAG TTG GAA CCA ATG AGC CTC AAG GAT
 Q  R  H  Y  L  G  E  E  L  E  P  M  S  L  K  D>         136

                         411
CTC CAA AAT CTG GAG CAG CAG CTT GAG ACT GCT CTT AAG CAC ATT CGC
 L  Q  N  L  E  Q  Q  L  E  T  A  L  K  H  I  R>         152

                                     471
TCC AGA AAA AAT CAA CTC ATG AAT GAG TCC CTC AAC CAC CTC CAA AGA
 S  R  K  N  Q  L  M  N  E  S  L  N  H  L  Q  R>         168

AAG GAG AAG GAG ATA CAG GAG GAA AAC AGC ATG CTT ACC AAA CAG ATA
 K  E  K  E  I  Q  E  E  N  S  M  L  T  K  Q  I>         184

      531
AAG GAG AGG GAA AAC ATC CTA AAG ACA AAA CAA ACC CAA TGT GAG CAG
 K  E  R  E  N  I  L  K  T  K  Q  T  Q  C  E  Q>         200

             591
CTG AAC CGC AGC GTC GAC GAT GTA CCA CAG CCA CAA CCA TTT CAA CAC
```

FIG 5A

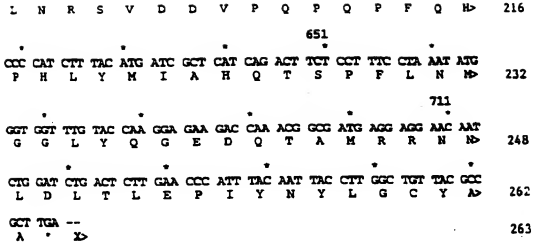
L N R S V D D V P Q P Q P F Q H> 216
651
CCC CAT CTT TAC ATG ATC GCT CAT CAG ACT TCT CCT TTC CTA AAT ATG
P H L Y M I A H Q T S P F L N M> 232
711
GGT GGT TTG TAC CAA GGA GAA GAC CAA ACG GCG ATG AGG AGG AAC AAT
G G L Y Q G E D Q T A M R R N N> 248
CTG GAT CTG ACT CTT GAA CCC ATT TAC AAT TAC CTT GGC TGT TAC GCC
L D L T L E P I Y N Y L G C Y A> 262
GCT TGA --
A * X> 263

FIG. 5B

```
ATG GGA AGG GGT AGG GTT GAA ATG AAG AGG ATA GAG AAC AAG ATC AAC
 M   G   R   G   R   V   E   M   K   R   I   E   N   K   I   N      16

          60
CGA CAA GTG ACG TTT TCG AAA AGA AGA GCT GGT CTT TTG AAG AAA GCC
 R   Q   V   T   F   S   K   R   R   A   G   L   L   K   K   A      32

                  120
CAT GAG ATC TCG ATC CTT TGT GAT GCT GAG GTT TCC CTT ATT GTC TTC
 H   E   I   S   I   L   C   D   A   E   V   S   L   I   V   F      48

                          180
TCC CAT AAG GGG AAA CTG TTC GAG TAC TCG TCT GAA TCT TGC ATG GAG
 S   H   K   G   K   L   F   E   Y   S   S   E   S   C   M   E      64

                                  240
AAG GTA CTA GAA CAC TAC GAG AGG TAC TCT TAC GCC GAG AAA CAG CTA
 K   V   L   E   H   Y   E   R   Y   S   Y   A   E   K   Q   L      80

AAA GTT CCA GAC TCT CAC GTC AAT GCA CAA ACG AAC TGG TCA GTG GAA
 K   V   P   D   S   H   V   N   A   Q   T   N   W   S   V   E      96

          300
TAT AGC AGG CTT AAG GCT AAG ATT GAG CTT TTG GAG AGA AAC CAA AGG
 Y   S   R   L   K   A   K   I   E   L   L   E   R   N   Q   R     112

                  360
CAT TAT CTG GGC GAA GAT TTA GAA TCA ATC AGC ATA AAG GAG CTA CAG
 H   Y   L   G   E   D   L   E   S   I   S   I   K   E   L   Q     128

                          420
AAT CTG GAG CAG CAG CTT GAC ACT TCT CTT AAA CAT ATT CGC TCG AGA
 N   L   E   Q   Q   L   D   T   S   L   K   H   I   R   S   R     144

                                  480
AAA AAT CAA CTA ATG CAC GAG TCC CTC AAC CAC CTC CAA AGA AAG GAG
 K   N   Q   L   M   H   E   S   L   N   H   L   Q   R   K   E     160

AAA GAA ATA CTG GAG GAA AAC AGC ATG CTT GCC AAA CAG ATA AGG GAG
 K   E   I   L   E   E   N   S   M   L   A   K   Q   I   R   E     176

          540
AGG GAG AGT ATC CTA AGG ACA CAT CAA AAC CAA TCA GAG CAG CAA AAC
 R   E   S   I   L   R   T   H   Q   N   Q   S   E   Q   Q   N     192
```

FIG. 6A

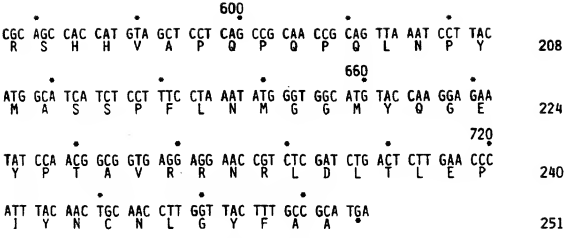
600
CGC AGC CAC CAT GTA GCT CCT CAG CCG CAA CCG CAG TTA AAT CCT TAC
R S H H V A P Q P Q P Q L N P Y 208
660
ATG GCA TCA TCT CCT TTC CTA AAT ATG GGT GGC ATG TAC CAA GGA GAA
M A S S P F L N M G G M Y Q G E 224
720
TAT CCA ACG GCG GTG AGG AGG AAC CGT CTC GAT CTG ACT CTT GAA CCC
Y P T A V R R N R L D L T L E P 240
ATT TAC AAC TGC AAC CTT GGT TAC TTT GCC GCA TGA
I Y N C N L G Y F A A 251

FIG. 6B

```
ATG GGA AGG GGT AGG GTT GAA ATG AAG AGG ATA GAG AAC AAG ATC AAC
 M   G   R   G   R   V   E   M   K   R   I   E   N   K   I   N      16

          60
AGA CAA GTG ACG TTT TCG AAA AGA AGA GCT GGT CTT TTG AAG AAA GCC
 R   Q   V   T   F   S   K   R   R   A   G   L   L   K   K   A      32

                  120
CAT GAG ATC TCG ATT CTT TGT GAT GCT GAG GTT TCC CTT ATT GTC TTC
 H   E   I   S   I   L   C   D   A   E   V   S   L   I   V   F      48

                          180
TCC CAT AAG GGG AAA CTG TTC GAG TAC TCG TCT GAA TCT TGC ATG GAG
 S   H   K   G   K   L   F   E   Y   S   S   E   S   C   M   E      64

                                  240
AAG GTA CTA GAA CGC TAC GAG AGG TAC TCT TAC GCC GAG AAA CAG CTA
 K   V   L   E   R   Y   E   R   Y   S   Y   A   E   K   Q   L      80

AAA GCT CCA GAC TCT CAC GTC AAT GCA CAA ACG AAC TGG TCA ATG GAA
 K   A   P   D   S   H   V   N   A   Q   T   N   W   S   M   E      96

          300
TAT AGC AGG CTT AAG GCT AAG ATT GAG CTT TGG GAG AGG AAC CAA AGG
 Y   S   R   L   K   A   K   I   E   L   W   E   R   N   Q   R     112

                  360
CAT TAT CTG GGA GAA GAT TTA GAA TCA ATC AGC ATA AAG GAG CTA CAG
 H   Y   L   G   E   D   L   E   S   I   S   I   K   E   L   Q     128

                          420
AAT CTG GAG CAG CAG CTT GAC ACT TCT CTT AAA CAT ATT CGC TCC AGA
 N   L   E   Q   Q   L   D   T   S   L   K   H   I   R   S   R     144

                                  480
AAA AAT CAA CTA ATG CAC TAG T CCCTCA ACCACCTCCA AAGAAAGGAG
 K   N   Q   L   M   H   *   X                                     150

                                          540
AAAGAAATAC TGGAGGAAAA CAGCATGCTT GCCAAACAGA TAAAGGAGAG GGAGAGTATC

                                          600
CTAAGGACAC ATCAAAACCA ATCAGAGCAG CAAAACCGCA GCCACCATGT AGCTCCTCAG

                                          660
CCGCAACCGC AGTTAAATCC TTACATGGCA TCATCTCCTT TCCTAAATAT GGGTGGCATG

                                          720
TACCAAGGAG AATATCCAAC GGCGGTGAGG AGGAACCGTC TCGATCTGAC TCTTGAACCC

ATTTACAACT GCAACCTTGG TTACTTTGCC GCATGA
```

FIG. 7

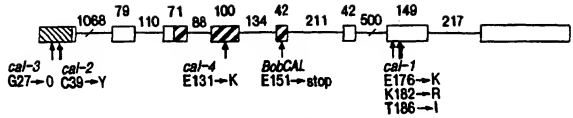

FIG. 8A

```
CAL     MGRGRVLLKRIKNKINRQVTFSKRRTGLLKKAQKISVLCDAKVSLIVFSK      50
BoCAL        M                  A    H   I
BobCAL       M                  A    H   I
AP1         Q                   A    H
                                                    A V

CAL     KGKLFEYSSESCMEKVLERYERYSYAERQLIAPDSHVNAQTNWSMEYSRL     100
BoCAL                 H              K  KV
BobCAL                               K  K
AP1           TD      I                   E D  --        N

CAL     KAKIELLERNORHYLGEELEPMSLKDLONLEQQLETALKHIRSRKNQLMY     150
BoCAL                 D  S  I  E              D  S             H
BobCAL        W       D  S  I  E              D                H
AP1                   D  QA  P  E              D        T       Y

CAL     ESLNHLQRKEKEIQEENSMLTKQIKERENILKTKQTQCEQLNRSVDDVPQ     200
BoCAL             L    V A   R   S  R H N S  Q    HHVA
BobCAL  *
AP1      I E  K   A   Q    S        K  RAQ E WD Q QGHNMP -

CAL     PQPFQHPHL---YMIAHQTSPFLNMGGLYQGEDQTAMRRNNLDLTLEPIY    247
BoCAL     QLN YM    -----AS          M    YP  V   R
BobCAL
AP1      L P QHQIQHP  LS  P             ED PM     D E     V

CAL     NY-LGCYAA*                                             255
BoCAL    CN  YF
BobCAL
AP1      CN   F
```

FIG. 8B

```
AAAGCAATCTGCTCAAAAGAGTAAAGAAAAGAAAAAAAGAAGTGATAGAGAGAGAGAAAAATAGATTATGGATCCTGAAGGTTTCACGAGTGGCTT   100
                                                                  M  D  P  E  G  F  T  S  G  L          10

ATTCCGGTGGAACCCAACGAGAGCATTGGTTCAAGCACCACCTCCGGTTCCACCTCCGCTGCAGCAACAGCCGGTGACACCGCAACGGCTGCTTTTGGG   200
 F  R  W  N  P  T  R  A  L  V  Q  A  P  P  P  V  P  P  L  Q  Q  Q  P  V  T  P  Q  T  A  A  F  G        43

ATGCGACTTGGTGGTTTAGAGGGACTATTCGGTCCATACGGTATACGTTCTACACGGCGGCGAAGATACGCGGAGTTACGTTTTACGGCGAGCACGCTTG   300
M  R  L  G  G  L  E  G  L  F  G  P  Y  G  I  R  F  Y  T  A  A  K  I  A  E  L  G  F  T  A  S  T  L  V     77

TGGGTATGAAGGACGAGGAGCTTGAAGAGATGATGATAGTCTCTCATATCTTTCGTTGGGAGCTTCTTGTTGGTGAACGGTACGGTATCAAAGCTGC   400
 G  H  K  D  E  E  L  E  E  M  M  M  S  L  S  H  I  F  R  W  E  L  L  V  G  E  R  Y  G  I  K  A  A     110

CGTTAGAGCTGAACGGAGACGATTGCAAGAAGGAGGAAGAGGAAATCTTCTAGACGCCGTCATTTGCTACTCTCCGCCGCTGGTGATTCCGGTACTCAT   500
 V  R  A  E  R  R  R  L  Q  E  E  E  E  E  E  S  S  R  R  R  H  L  L  S  A  A  G  D  S  G  T  H        143

CACGCTCTTGATGCTCCTCTCCCAAGAAGATGATTGGACAGGGTTATCTGAGGAACCGGTGCAGCAACAAGACCAGACTGATGCGGCGGGGAATAACGGCG   600
H  A  L  D  A  L  S  Q  E  E  D  D  W  T  G  L  S  E  E  P  V  Q  Q  Q  D  Q  T  D  A  A  G  N  N  G  G   177

GAGGAGGAAGTGGTTACTGGGACGCAGGTCAAGGAAAGATGAAGAAGCAACAGCAGCAGAGACGGAGAAGAAAGAAACCAATGCTGACGTCAGTGGAAACCGA   700
 G  G  S  G  Y  W  D  A  G  Q  G  K  M  K  K  Q  Q  Q  Q  R  R  R  K  K  P  M  L  T  S  V  E  T  D     210

CGAAGACGTCAACGAAGGTGAGGATGACGACGGGATGGATAACGGCAACGGAGGTAGTGGTTTGGGGACAGAGAGACAGAGGGAGCATCCGTTTTATCGTA   800
E  D  V  N  E  G  E  D  D  D  G  M  D  N  G  N  G  G  S  G  L  G  T  E  R  Q  R  E  H  P  F  I  V        243
```

FIG. 9A

```
ACGGAGCCTGGGGAAGTGGCACGTGGCAAAAAGAACGGCTTAGATTATCTGTTCCACTTGTACGAACAATGCCGTGAGTTCCTTCTTCAGGTCCAGACAA   900
 T  E  P  G  E  V  A  R  G  K  K  N  G  L  D  Y  L  F  H  L  Y  E  Q  C  R  E  F  L  L  Q  V  Q  T  I   277

TTGCTAAAGACCGTGGCGAAAAATGCCCCACCAAGGTGACGAACCAAGTATTCAGGTACGCGAAGAAATCAGGAGCGAGTTACATAAACAAGCCTAAAAT  1000
   A  K  D  R  G  E  K  C  P  T  K  V  T  N  Q  V  F  R  Y  A  K  K  S  G  A  S  Y  I  N  K  P  K  M   310

GCGACACTACGTTCACTGTTACGCTCTCCACTGCCTAGACGAAGAAGCTTCAAATGCTCTCAGAAGAGCGTTTAAAGAACGCGGTGAGAACGTTGGCTCA  1100
 R  H  Y  V  H  C  Y  A  L  H  C  L  D  E  E  A  S  N  A  L  R  R  A  F  K  E  R  G  E  N  V  G  S      343

TGGCGTCAGGCTTGTTACAAGCCACTTGTGAACATCGCTTGTCGTCATGGCTGGGATATAGACGCCGTCTTTAACGCTCATCCTCGTCTCTCTATTTGGT  1200
W  R  Q  A  C  Y  K  P  L  V  N  I  A  C  R  H  G  W  D  I  D  A  V  F  N  A  H  P  R  L  S  I  W  Y   377

ATGTTCCAACAAAGCTGCGTCAGCTTTGCCATTTGGAGCGGAACAATGCGGTTGCTGCGGCTGCGGCTTTAGTTGGCGGTATTAGCTGTACCGGATCGTC  1300
   V  P  T  K  L  R  Q  L  C  H  L  E  R  N  N  A  V  A  A  A  A  A  L  V  G  G  I  S  C  T  G  S  S   410

GACGTCTGGACGTGGTGGATGCGGCGGCGACGACTTGCGTTTCTAGTTTGGTTTGGGTAGTTGTGGTTTGTTTAGTCGTTATCCTAATTAACTATTAGTC  1400
 T  S  G  R  G  G  C  G  G  D  D  L  R  F  stop                                                          424

TTTAATTTAGTCTTCTTGGCTAATTTATTTTTCTTTTTTTGTCAAAACCTTTAATTTGTTATGGCTAATTTGTTATACACGCAGTTTTCTTAATGCGTTA  1500
```

FIG. 9B

```
                                                                  60
GAATTCCCCG GATCTCCATA TACATATCAT ACATATATAT AGTATACTAT  CTTTAGACTG ATTTCTCTAT ACACTATCTT TTAACTTATG TATCGTTTCA
                                                                 120
AAACTCAGGA CGTACATGTT TTAAATTTGG TTATATAACC ACGACCATTT  CAAGTATATA TGTCATACCA TACCAGATTT AATATAACTT CTATGAAGAA
                                                                 180
AATACATAAA GTTGGATTAA AATGCAAGTG ACATCTTTTT AGCATAGGTT  CATTTGGCAT AGAAGAAATA TATAACTAAA AATGAACTTT AACTTAAATA
                                                                 240
GATTTTACTA TATTACAATT TTTCTTTTTA CATGGTCTAA TTTATTTTTC  TAAAATTAGT ATGATTGTTG TTTTGATGAA ACAATAATAC CGTAAGCAAT
                                                                 300
AGTTGCTAAA AGATGTCCAA ATATTTATAA ATTACAAAGT AAATCAAATA  AGGAAGAAGA CACGTGGAAA ACACCAAATA AGAGAAGAAA TGGAAAAAAC
                                                                 360
AGAAAGAAAT TTTTTAACAA GAAAAATCAA TTAGTCCTCA AACCTGAGAT  ATTTAAAGTA ATCAACTAAA ACAGGAACAC TTGACTAACA AAGAAATTTG
                                                                 420
AAATGTGGTC CAACTTTCAC TTAATTATAT TATTTTCTCT AAGGCTTATG  CAATATATGC CTTAAGCAAA TGCCGAATCT GTTTTTTTTT TTTGTTATTG
                                                                 480
GATATTGACT GAAAATAAGG GGTTTTTTCA CACTTGAAGA TCTCAAAAGA  GAAAACTATT ACAACGGAAA TTCATTGTAA AAGAAGTGAT TAAGCAAATT
                                                                 540
```

FIG 10A

GAGCAAAGGT TTTTATGTGG TTTATTTCAT TATATGATTG ACATCAAATT

900

GTATATATAT GGTTGTTTTA TTTAACAATA TATATGGATA TAACGTACAA

ACTAAATATG TTTGATTGAC GAAAAAAAAT ATATGTATGT TTGATTAACA

960

ACATAGCACA TATCAACTGA TTTTTGTCCT GATCATCTAC AACTTAATAA

1020

GAACACACAA CATTGAAAAA ATCTTTGACA AAATACTATT TTTGGGTTTG

1080

AAATTTTGAA TACTTACAAT TATCTTCTCG ATCTTCCTCT CTTTCCTTAA

1140

ATCCTGCGTA CAAATCCGTC GACGCAATAC ATTACACAGT TGTCAATTGG

1200

TTCTCAGCTC TACCAAAAAC ATCTATTGCC AAAAGAAAGG TCTATTTGTA

CTTCACTGTT ACAGCTGAGA ACATTAAATA TAATAAGCAA ATTTGATAAA

1260

ACAAAGGGTT CTCACCTTAT TCCAAAAGAA TAGTGTAAAA TAGGGTAATA

1320

GAGAAATGTT AATAAAAGGA AATTAAAAAT AGATATTTTG GTTGGGTTCA

1380

GATTTTGTTT CGTAGATCTA CAGGGAAATC TCCGCCGTCA ATGCAAAGCG

1440

AAGGTGACAC TTGGGGAAGG ACCAGTGGTC GTACAATGTT ACTTACCCAT

1500

TTCTCTTCAC GAGACGTCGA TAATCAAATT GTTTATTTTC ATATTTTTAA

GTCCGCAGTT TTATTAAAAA ATCATGGACC CGACATTAGT ACGAGATATA

1560

CCAATGAGAA GTCGACACGC AAATCCTAAA GAAACCACTG TGGTTTTTGC

1620

AAACAAGAGA AACCAGCTTT AGCTTTTCCC TAAAACCACT CTTACCCAAA

FIG 10B

```
                                                1680
TCTCTCCATA AATAAAGATC CGGAGACTCA AACACAAGTC TTTTTATAAA

                                                1740
GGAAAGAAAG AAAAACTTTC CTAATTGGTT CATACCAAAG TCTGAGCTCT

                                                1800
TCTTTATATC TCTCTTGTAG TTTCTTATTG GGGGTCTTTG TTTTGTTTGG

TTCTTTTAGA GTAAGAAGTT TCTTAAAAAA GGATCAAAAA TGGGAAGGGG
1860
TAGGGTTCAA TTGAAGAGGA TAGAGAACAA GATCAATAGA CAAGTGACAT
                1920
TCTCGAAAAG AAGAGCTGGT CTTTTGAAGA AAGCTCATGA GATCTCTGTT
                                        1980
CTCTGTGATG CTGAAGTTGC TCTTGTTGTC TTCTCCCATA AGGGGAAACT
                                                2040
CTTCGAATAC TCCACTGATT CTTGGTAACT TCAACTAATT CTTTACTTTT
                                                2100
AAAAAAATCT TTTAATCTGC TACTTTATAT AGTTTTTTTC CCCC-----GG

TCTATGATTC ATACTGTTTT GTTATTATAA AGGTATCATA GAGATCGGTA
2160
CTTGATTTGT TATAGGAAAT CTTGGTTTAA TTGCATAAAA CCATCATTAG
                2220
ATTTATCCTA AAATGTGATG ATATTTTGGT CACATCTCCA TATTATTTAT
                                        2280
ATAATAAAAT GATAATTGGT TGATGATAAA GCTAACCCCA ATTCTGTGAA
                                                2340
ATGATCAGTA TGGAGAAGAT ACTTGAACGC TATGAGAGGT ACTCTTACGC
                                                2400
CGAAAGACAG CTTATTGCAC CTGAGTCCGA CGTCAATGTA TTTCAATAAA

TATTTCTCCT TTTAATCCAC ATATATATTA TATCAATCTA TTTGTAGTAT
2460
```

FIG. 10C

```
TGATGAATTT TATTTGTATA AAACTTCTGG TACACAGACA AACTGGTCGA

                2520
TGGCGTATAA CAGGCTTAAG GCTAAGATTG AGCTTTTGGA GAGAAACCAG

                2580
AGGTACACAT TTACACTCAT CACATTTCTA TCTAGAAAAT CGATCGGGTT

                2640
CCATTTTAAA GTAAGTTAAA ATTCATTGAT GCTATTGAAA TTCAGGCATT

                2700
ATCTTGGGGA AGACTTGCAA GCAATGAGCC CTAAAGAGCT TCAGAATCTG

GAGCAGCAGC TTGACACTGC TCTTAAGCAC ATCCGCACTA GAAAGTATT

2760
GCCTTCTGCT ATTTCGTTGA ACATATCTAT ATAACTTAAA CGTTTACAAG

                2820
TGTTATTATA ATGTGAACAT TGAAATACAT ATGTGTATGT ATCAATATAT

                2880
ATATCAGTAA TCAATATCAA TTTGATATGT CTATAGGTTG GTTCGAATGT

                2940
ATGAGTTATG TTGTGTATTT TAAGACTCCA TATTACTTAA AGTAATGGGT

                3000
TGTTAATGTT GATGTAGTGT TATGCAGAAC CAACTTATGT ACGAGTCCAT

CAATGAGCTC CAAAAAAAGG TATGTAAAAC CCCTATCAAA TGTATGTCTT

3060
ATAGAGAAAC GTATAGGAAA GCTAATTAAC AATCGTGCCG TTTCGGAATG

                3120
ACAGGAGAAG GCCATACAGG AGCAAAACAG CATGCTTTCT AAACAGGAAC

                3180
ACATGTCATC ATTTCTCTTT CATCAACATG TTGTCCATTG CATTACTGTT

                3240
ACCTTCCACT GTTCTGCTCC ACACTTCCAG CCAAGCTATA CCTACGATAT

                3300
CTTCATATCT CCACTTAACT TCGGCACCAT TAAATAAAAA TAGAAAATCT
```

FIG. 10D

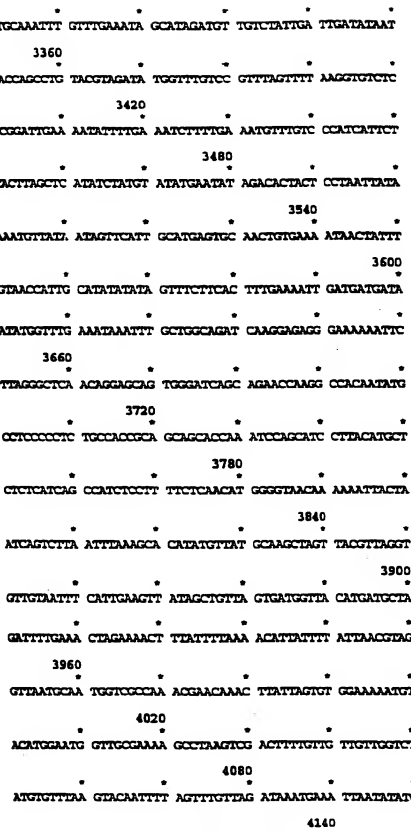
TTGCAAATTT GTTTGAAATA GCATAGATGT TGTCTATTGA TTGATATAAT
3360
CACCAGCCTG TACGTAGATA TGGTTTGTCC GTTTAGTTTT AAGGTGTCTC
3420
TCGGATTGAA AATATTTTGA AATCTTTTGA AATGTTTGTC CCATCATTCT
3480
TACTTAGCTC ATATCTATGT ATATGAATAT AGACACTACT CCTAATTATA
3540
AAATGTTATA ATAGTTCATT GCATGAGTGC AACTGTGAAA ATAACTATTT
3600
GTAACCATTG CATATATATA GTTTCTTCAC TTTGAAAATT GATGATGATA
ATATGGTTTG AAATAAATTT GCTGGCAGAT CAAGGAGAGG GAAAAAATTC
3660
TTAGGGCTCA ACAGGAGCAG TGGGATCAGC AGAACCAAGG CCACAATATG
3720
CCTCCCCCTC TGCCACCGCA GCAGCACCAA ATCCAGCATC CTTACATGCT
3780
CTCTCATCAG CCATCTCCTT TTCTCAACAT GGGGTAACAA AAAATTACTA
3840
ATCAGTCTTA ATTTAAAGCA CATATGTTAT GCAAGCTAGT TACGTTAGGT
3900
GTTGTAATTT CATTGAAGTT ATAGCTGTTA GTGATGGTTA CATGATGCTA
GATTTTGAAA CTAGAAAACT TTATTTTAAA ACATTATTTT ATTAACGTAG
3960
GTTAATGCAA TGGTCGCCAA ACGAACAAAC TTATTAGTGT GGAAAAATGT
4020
ACATGGAATG GTTGCGAAAA GCCTAAGTCG ACTTTTGTTG TTGTTGGTCT
4080
ATGTAGTTAA GTACAATTTT AGTTTGTTAG ATAAATGAAA TTAATATATC
4140

FIG. 10E

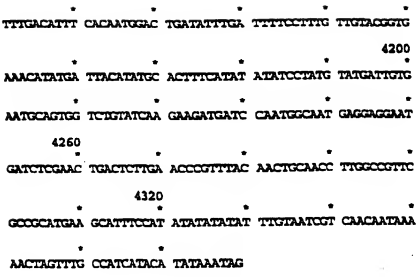
TTTGACATTT CACAATGGAC TGATATTTGA TTTTCCTTTG TTGTACGGTG
4200
AAACATATGA TTACATATGC ACTTTCATAT ATATCCTATG TATGATTGTG
AATGCAGTGG TCTGTATCAA GAAGATGATC CAATGGCAAT GAGGAGGAAT
4260
GATCTCGAAC TGACTCTTGA ACCCGTTTAC AACTGCAACC TTGGCCGTTC
4320
GCCGCATGAA GCATTTCCAT ATATATATAT TTGTAATCGT CAACAATAAA
AACTAGTTTG CCATCATACA TATAAATAG

FIG. 10F

```
         *          *          *          *          *
GCACCTGAGT CCGACTCCAA TGTAAACCAA TTTCTCTCCA TTAACTTATA
        60
         *          *          *          *          *
TAAATTAAAT ATTATTTCAG TATTAGTGAT ATATACTTAT CTGTATTAAA
                   120
         *          *          *          *          *
CTTGTGAGAT ATAGACGAAC TGGTCGATGG AGTATAATAG GCTTAAGGCT
                              180
         *          *          *          *          *
AAGATTGAGC TTTTGGAGAG AAACCAGAGG TACATTTTCA TTCATCATTT
                                         240
         *          *          *          *          *
ATATTAATAG ATGAAATATC AAACAGGATT AATGTTAGTT AAAAATGCAT
                                                    300
         *          *          *          *          *
GATTACTTAT AAGAAAATGA TGCATTTAAA TAACAAAAAA ATGCATCGAT
         *          *          *          *          *
GCTCTATTGA AATTTAGGCA CTATCTTGGG GAAGACTTGC AAGCAATGAG
       360
         *          *          *          *          *
CCCTAAGGAA CTCCAGAATC TAGAGCAACA GCTTGATACT GCTCTTAAGC
                   420
         *          *          *          *          *
ACATCCGCTC TAGAAAAGTA TGAATCCTCC TATTTCTTTA ATTAACATGT
                              480
         *          *          *          *          *
ATACAACTTA AACACATATT ATTTTATTAT TCAATACATA TATATGAATA
                                         540
         *          *          *          *          *
GTACATATGT GATTTTATTG GTTGGATATA AAAGATCAAT CACGTCGATT
                                                    600
         *          *          *          *          *
AGATGTATGA CTTTTTAAAG AATTAGTATA TAGAGTATGA TTAGTCAATG
         *          *          *          *          *
TAATGGTACG TACGTTTATG CAGAACCAAC TTATGTACGA CTCCATCAAT
       660
         *          *          *          *          *
GAGCTCCAAA GAAAGGTATG TATAAACCCT ATCAAATTGA CGTTTACATA
                   720
         *          *          *          *          *
GAATAACTGC GTGTAAGAAT CCTATAGGGG AGCTAACAAT CGTGCCGTTT
                              780
         *          *          *          *          *
TGGAAATGAC AGGAGAAAGC CATACAGGAA CAAAACAGCA TGCTTTCCAA
                                         840
         *          *          *          *          *
```

FIG. IIA

SUBSTITUTE SHEET (RULE 26)

GCAGGTGCCA TTTGTCATTA TTTTTATATC GTCAAAATGT TTTCTATTGT

900

AGTACTGTTA GCTTCCACTG TTCTACTCCA CACTTCAAGC CAAGCTATAC

CTACCTACGA CTACGAGATT CTCCACATAT TTCTCCACTT AGCTTCGGCA

960

CCACTATAAC TAAAATATAG ATAAAATATC ATTTTTATAG TCTATGATTG

1020

ATATACTCGT CAGCCAGTAC GTAGTTGGGT ATTTGCCCGT TTAGTTTTAA

1080

GGTTCTTTTC CGGATTGAAA ATATTT---- -ACCCTACCT TTGATGCTAT

1140

TATATGTATA TCTATTTAGA AGTCGTGGCT TTGAAAATTG ATGATGATAT

1200

GTATGGTATA AGTTGGTAAC AAACTGGTGT GTGAAATTGA AACTTGTCAG

ATTAAGGAGA GGGAAAACGT TCTTAGGGCG CAACAAGAGC AATGGGACGA

1260

GCAGAACCAT GGCCATATAT GCCTCCGCCT CCACCCCCGC AGCAGCATCA

1320

AATCCAGCAT CCTTACATGC TCTCTCATCA GCCATCTCCT TTTCTCAACA

1380

TGGGGTAGTT AAAAATTCGT TCCTCTTACT TTCAAGTCAT ATGTGTATAT

1440

ATACAAGATA GTTAGGTGTT ATAAGTCCAG TGAGTTAGGT TGTGTTAGTG

1500

ATGGTTAGAT GTCTAGATTG TGAATTACAA GTACTAAGAT TTTTCAGTTA

TATAATTAAC GTATTGATCA TCAATCAAAT GGTCGTAAAA AAACAGACTT

1560

ATATTTTTGG GAAAGTAGAT GGAATGGCTG CTAAAAGTCT AAGAAACCTT

1620

TGGGAGCAGG TCGTATTTAT TGTTGTTCAA ATTAAACTTG AGGTAGTTAG

FIG. IIB

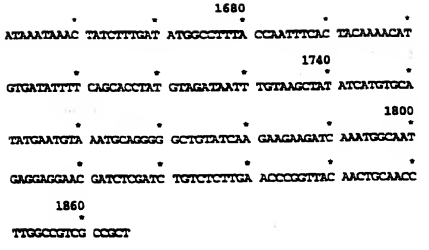
1680
ATAAATAAAC TATCTTTGAT ATGGCCTTTA CCAATTTCAC TACAAAACAT
1740
GTGATATTTT CAGCACCTAT GTAGATAATT TGTAAGCTAT ATCATGTGCA
1800
TATGAATGTA AATGCAGGGG GCTGTATCAA GAAGAAGATC AAATGGCAAT
GAGGAGGAAC GATCTCGATC TGTCTCTTGA ACCCCGTTAC AACTGCAACC
1860
TTGGCCGTCG CCGCT

FIG. IIC

```
GAGCTCTTCT TTATATCTCT TCTTGTAGTT TCTTGTTTCG TTTGGTTCTC
       60
TTAGAGGAAA TAGTTCCTTT AAAAGGGATA AAAATGGGAA GGGGTAGGGT
                  120
TCAGTTGAAG AGGATAGAAA ACAAGATCAA TAGACAAGTG ACATTCTCGA
                             180
AAAGAAGAGC TGGTCTTATG AAGAAAGCTC ATGAGATCTC TGTTCTGTGT
                                        240
GATGCTGAAG TTGCGCTTGT TGTCTTCTCC CATAAGGGGA AACTCTTTGA
                                                   300
ATACCCCACT GATTCTTGGT AACTTTCTCA TTTAAGAAAC AAAA---TAC
CCTAAGATTG TATTTTACAT GATCATTTAC TTGTTTTACA CAGTATATAC
       360
TCTATGTATA TAATATGATC ATAAATTGTT GATGATAAGA AGCTAGCCCT
                  420
AATTCTGTGA ATTGAACAGT ATGGAGGAGA TACTTGAACG CTATGAGAGA
                             480
TACTCTTACG CCGAGAGACA GCTTATAGCA CCTGAGTCCG ACTCCAATGT
                                        540
AAACCAATTT CTCTCCATTA ACTTATATAA ATTAAATATT ATTTCAGTAT
                                                   600
TAGTGATATA TACTTATCTG TATTAAACTT GTGAGATATA GACGAACTGG
TCGATGGAGT ATAATAGGCT TAAGGCTAAG ATTGAGCTTT TGGAGAGAAA
       660
CCAGAGGTAC ATTTTCATTC ATCATTTATA TATATGATGA AATATCAAAC
                  720
AGGATTAATG TTAGTTAAAA ATGCATGATT ACTTATAAAA AAATGATGCA
                             780
TTTAAATAAC AAAAAAATGC ATCGATGCTC TATTGAAATT TAGGCACTAT
                                        840
```

FIG. 12A

CTTGGGGAAG ACTTGCAAGC AATGAGCCCT AAGGAACTCC AGAATCTAGA

900

GCAACAGCTT GATACTGCTC TTAAGCACAT CCGCTCTAGA AAAGTATGAA

TCCTCCTATT TCTTTAATTA ACATGTATAC AACTTAAACA CATATTATTT

960

TATTATTCAA ATACATATAT ATAAATAGTA CATATGTGAT TTTATTGGTT

1020

GGATTTGAAA AGATCAATCA CGTCGATTAG AATGTATGAC TTTTTAAAGA

1080

ATTAGTATAT AGAGTATGAT TAGTCAATGT AATGGATCGT TTATGCAGAA

1140

CCAACTTATG TACGACTCCA TCAATGAGCT CCAAAGAAAG GTATGTATAA

1200

ACCCTATCAA ATTGACGTTT ACATAGAATA ACTGCGTGTA AGAATCCTAT

AGGGGAGCTA AAAATCGTGC CGTTTTGGAA ATGACAGGAG AAAGCCATAC

1260

AGGAACAAAA CAGCATGCTT TCCAAGCAGG TGCCATTTGT CATTATTTTT

1320

ATTTCGTCAA AATGTTTTCT ATTGTAGATC TGTTAGCTTC CACTGTTCTC

1380

ACCACACTTC AAGCCAAGCT ATACCTACCT ACGACTAC-- --CCTACATTT

1440

GATGCTATTT ATATGTATAT CTATTTAGAA GTCGTGGCTT TGAAAATTGA

1500

TGATGATATG GTATGGTATA AGTTGGTAAC AAACTGGTGT GTGAAATTGA

AACTTGTCAG ATTAAGGAGA GGGAAAACGT TCTTAGGGCG CAACAAGAGC

1560

AATGGGACGA GCAGAACCAT GGCCATAATA TGCCTCCGCC TCCACCCCCG

1620

CAGCAGCATC AAATCCAGCA TCCTTACATG CTCTCTCATC AGCCATCTCC

FIG 12B

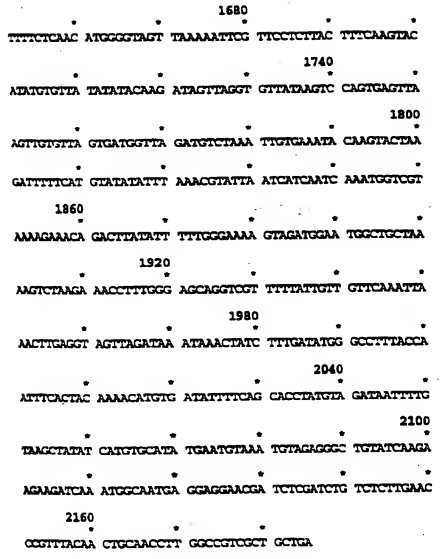
1680
TTTTCTCAAC ATGGGGTAGT TAAAAATTCG TTCCTCTTAC TTTCAAGTAC
1740
ATATGTGTTA TATATACAAG ATAGTTAGGT GTTATAAGTC CAGTGAGTTA
1800
AGTTGTGTTA GTGATGGTTA GATGTCTAAA TTGTGAAATA CAAGTACTAA
GATTTTTCAT GTATATATTT AAACGTATTA ATCATCAATC AAATGGTCGT
1860
AAAAGAAACA GACTTATATT TTTGGGAAAA GTAGATGGAA TGGCTGCTAA
1920
AAGTCTAAGA AACCTTTGGG AGCAGGTCGT TTTTATTGTT GTTCAAATTA
1980
AACTTGAGGT AGTTAGATAA ATAAACTATC TTTGATATGG GCCTTTACCA
2040
ATTTCACTAC AAAACATGTG ATATTTTCAG CACCTATGTA GATAATTTTG
2100
TAAGCTATAT CATGTGCATA TGAATGTAAA TGTAGAGGGC TGTATCAAGA
AGAAGATCAA ATGGCAATGA GGAGGAACGA TCTCGATCTG TCTCTTGAAC
2160
CCGTTTACAA CTGCAACCTT GGCCGTCGCT GCTGA

FIG. 12C

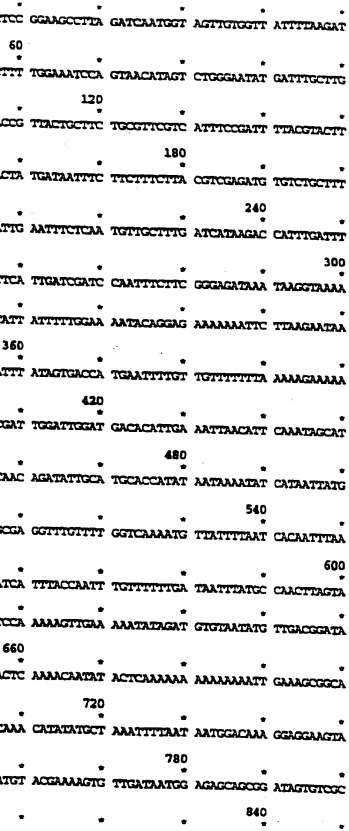
GGATCCCTCC GGAAGCCTTA GATCAATGGT AGTTGTGGTT ATTTTAAGAT
60
CAGATTCTTT TGGAAATCCA GTAACATAGT CTGGGAATAT GATTTGCTTG
120
TTGGTCACCG TTACTGCTTC TGCGTTCGTC ATTTCCGATT TTACGTACTT
180
TTGATCACTA TGATAATTTC TTCTTTCTTA CGTCGAGATG TGTCTGCTTT
240
TTGTAGATTG AATTTCTCAA TGTTGCTTTG ATCATAAGAC CATTTGATTT
300
CTTTCCTTCA TTGATCGATC CAATTTCTTC GGGAGATAAA TAAGGTAAAA
ATGGACTATT ATTTTTGGAA AATACAGGAG AAAAAAATTC TTAAGAATAA
360
AAGAGTATTT ATAGTGACCA TGAATTTTGT TGTTTTTTTA AAAAGAAAAA
420
AAAACTCGAT TGGATTGGAT GACACATTGA AATTAACATT CAAATAGCAT
480
CTTAGTTAAC AGATATTGCA TGCACCATAT AATAAAATAT CATAATTATG
540
TGTGATGCGA GGTTTGTTTT GGTCAAAATG TTATTTTAAT CACAATTTAA
600
TAACAGATCA TTTACCAATT TGTTTTTTGA TAATTTATGC CAACTTAGTA
AATTCATCCA AAAAGTTGAA AAATATAGAT GTGTAATATG TTGACGGATA
660
TACAACACTC AAAACAATAT ACTCAAAAAA AAAAAAAATT GAAAGCGGCA
720
ACGATTCAAA CATATATGCT AAATTTTAAT AATGGACAAA GGAGGAAGTA
780
CTGCATATGT ACGAAAAGTG TTGATAATGG AGAGCAGCGG ATAGTGTCGC
840

FIG. 13A

CAAGGGCACG AGCTTTAGAT TCTTTTAGTT TGCTCTAAAT GTTCTTCTTT

900

GGTACTTTTA ATTGCTTTAG TTGCTTGCTT CTTATCTCCA CATAAATAAA

TGGGGTAACC ATTTTCTCTC GTATCTTATT CCGATCTTTG GATCTATGTA

960

CGTACTACAT GAATAAATCG TGTTCAATAA GTTATTATCA TTTGGTCTGC

1020

TTAAAGTGAT CATGGTGTAT TAATCTATAA TACGTAGTTC TCTTAATTTA

1080

TTCCCTAGAA TTCCATCAAA GACAAATTTT AGCAAAAAGA AAAGTTGAGT

1140

ATATAATTTG CTTAGTAGTA CAAAAAAAAA CTTTATGGTA ATTTGTATTT

1200

TGGATATTTC CTTNATTAAC CCAAACTTCA AAATTAATTT TCTTCTGCTG

TATCTTTATA TCCAACGTGA AATCTATTGA CTCAACAAAA TACACAGTTG

1260

TCAATTGAAG TTCAACTCTA CCAAGAAACA TCTATATGTA CTTCACTGTT

1320

CTTACCGCCG AGCAATTAAA ACCTCTATAA CTACTTGGTT ACATTATTAC

1380

ATTTTTATTT ACAAAAAATA TATATCAACA ACCAATAATA TAGTTAGAAA

1440

ATGAAAGAAA ATTATTTAAG AAATATCCGC CGTCAATGCA AATCGAATGC

1500

GACACTTGGG GAAGCTCTGA AGTCTGTGGT CTGTGCATAT TTCACTTGTC

TAGCTAACCC ATTTTCACGT CACTAGACGT CGATAATCAA TTATTGTTAT

1560

TTTTTTTATC AATGTTCCAC TTATTGAAAA TTATATACGA GAAAACATAG

1620

ACTCGACATT AGGCAATGGA AGTCTAATCA GACCAATGAG AAGTCGACAA

FIG 13B

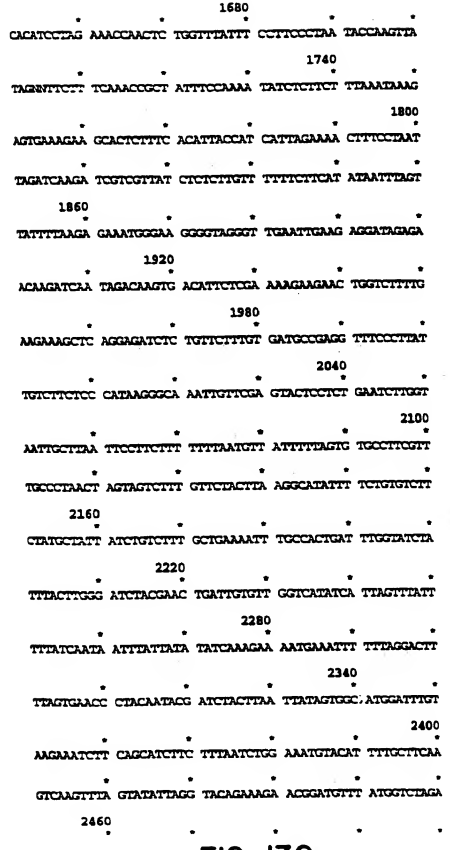

FIG. 13C

SUBSTITUTE SHEET (RULE 26)

CTAGGGTTTT TGCTTTTAGG AAAGCTATAC TTTTGCTTAA ATATCTTTAA

2520

GTTGCATTTT ATGAACACAC ACACACATAT ATATATATAT ATATTAGTAT

2580

ACCAATAATC TTAATTAAGT TTAGAAAGAA ACTCTTCATT TTTTCCCATT

2640

TAATAATGGT TTATAGCTAG GTATAGAGAA ACTGGAAATA AGTATGTGAC

2700

ATCTAAGTAT GGGGAGTCTT TGACCTCTGG GGATTAATGT AAAACAGATC

GTTCTTTTTT TTCTAAACAG TTCCTCCGTA CTGATGGTCA AACTTAACTT

2760

CAACAGTTCC TTTTAAACTT TTATAGGGTG CTTGAATACG TCTTGGGGTG

2820

TGGGGTTAGT GGCTCAACTG GTTTATTTAT TTTTAAAAAT GGTAGAAATC

2880

AGTACTGTTT CTAGCTAGGG TTTAGGCACA AAACTAGAGA TCATCTTTAT

2940

TCCATAATAG AAAGGAAGAA ACTAATGTTT AATGACATAG ATTAATTAGA

3000

TAACCCTACA TAATCAGATG CTATATGTTA TCACATATTT TGGGTGAATC

GTTAATTACG TTTGAAACAA GTGGCCTCTT GTGCTAGCTG ATAAGATAGT

3060

TGNGTATGCA ATTATATTGG TGGTTGAATC CAAACTAATT CTAACTCGTA

3120

AGCTTAATAT TTGTAGCATG GAGAAGGTAC TAGAACGCTA CGAGAGGTAT

3180

TCTTACGCCG AGAGACAGCT GATTGCACCT GACTCTCACG TTAATGTATG

3240

TTTAATGGTC TCCATCATAT ATTTGTGTAT ATTTTGAATC TTGCATGTGT

3300

TTTAACATAG CATATAACTG ATTATTGGCT TTCATGTTGG AAATTAATTG

FIG. 13D

```
                                                                    *
TGAAGGCACA GACGAACTGG TCAATGGAGT ATAGCAGGCT TAAGGCCAAG
      3360
       *          *          *          *          *
ATTGAGCTTT TGGAGAGAAA CCAAAGGTAC ATAGTACATT TAAATTTATT
            3420
       *          *          *          *          *
GTAGTAGTTA AATATTGAGG AATAACAGAA GAGAGAATGT TCTTAATTAA
                  3480
       *          *          *          *          *
CTAAATCATC ATAGGCATTA TCTGGGAGAA GAGTTGGAAC CAATGAGCCT
                        3540
       *          *          *          *          *
CAAGGATCTC CAAAATCTGG AGCAGCAGCT TGAGACTGCT CTTAAGCACA
                                                  3600
       *          *          *          *          *
TTCGCTCCAG AAAAGTGTGT AAATATATCC CACACTCTAT CTCTATGCAT
       *          *          *          *          *
AACTAACTTT GACTTTGTGT GGATGTATTA CATATAGTCA AATATTGTAT
      3660
       *          *          *          *          *
AGAGATTGTC TCATATAAAT AAATAATTTT TGGCCTTTTT GTATGCAGAA
            3720
       *          *          *          *          *
TCAACTCATG AATGAGTCCC TCAACCACCT CCAAAGAAAG GTAGCTAAGT
                  3780
       *          *          *          *          *
TAAAACCATT TTATCTCTCA AGTCCTGTGT GTATAGAGTC ATGACTTATA
                        3840
       *          *          *          *          *
TGTTAGAGAT ATAAATCTTT TAATAAATAA ATAACATATA GGTTATATAT
                                                  3900
       *          *          *          *          *
AATTCAGGTT AATATATTAT TAATTACTAG ATGTATATAT ACTTATATAG
       *          *          *          *          *
ATCATATAAA AAGAGAAATT GACAATGGTG TCATTTTTGT GGAAATGACA
      3960
       *          *          *          *          *
GGAGAAGGAG ATACAGGAGG AAAACAGCAT GCTTACCAAA CAGGTGATCA
            4020
       *          *          *          *          *
TTGTTTTTTG CATTTCTAAC TGTTTCACTA TTTACAATTC CACTGTTGAA
                  4080
       *          *          *          *          *
CTCCACTTCA ATCTCTACCT TAACGTACCA TCTCTCCACT TTCGGCCCCA
                                                  4140
```

FIG. 13E

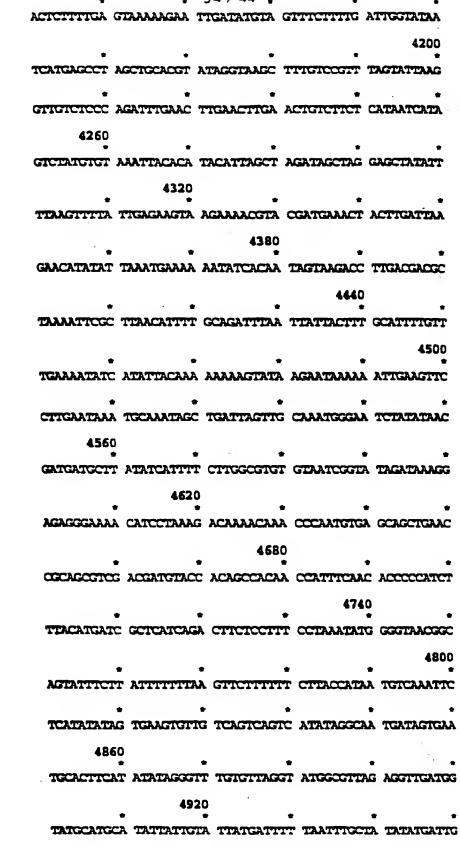

FIG. 13F

SUBSTITUTE SHEET (RULE 26)

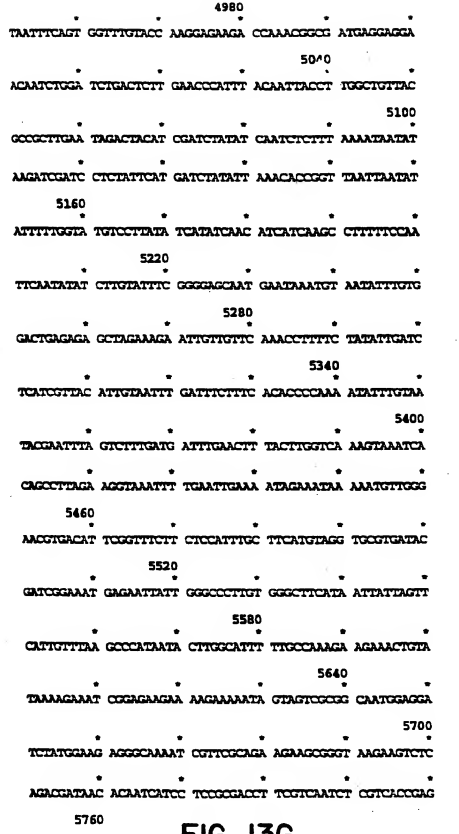
4980
TAATTTCAGT GGTTTGTACC AAGGAGAAGA CCAAACGGCG ATGAGGAGGA
5040
ACAATCTGGA TCTGACTCTT GAACCCATTT ACAATTACCT TGGCTGTTAC
5100
GCCGCTTGAA TAGACTACAT CGATCTATAT CAATCTCTTT AAAATAATAT
AAGATCGATC CTCTATTCAT GATCTATATT AAACACCGGT TAATTAATAT
5160
ATTTTTGGTA TGTCCTTATA TCATATCAAC ATCATCAAGC CTTTTTCCAA
5220
TTCAATATAT CTTGTATTTC GGGGAGCAAT GAATAAATGT AATATTTGTG
5280
GACTGAGAGA GCTAGAAAGA ATTGTTGTTC AAACCTTTTC TATATTGATC
5340
TCATCGTTAC ATTGTAATTT GATTTCTTTC ACACCCCAAA ATATTTGTAA
5400
TACGAATTTA GTCTTTGATG ATTTGAACTT TACTTGGTCA AAGTAAATCA
CAGCCTTAGA AGGTAAATTT TGAATTGAAA ATAGAAATAA AAATGTTGGG
5460
AACGTGACAT TCGGTTTCTT CTCCATTTGC TTCATGTAGG TCCGTGATAC
5520
GATCGGAAAT GAGAATTATT GGGCCCTTGT GGGCTTCATA ATTATTAGTT
5580
CATTGTTTAA GCCCATAATA CTTGGCATTT TTGCCAAAGA AGAAACTGTA
5640
TAAAAGAAAT CGGAGAAGAA AAGAAAAATA GTAGTCGCGG CAATGGAGGA
5700
TCTATGGAAG AGGGCAAAAT CGTTCGCAGA AGAAGCGGGT AAGAAGTCTC
AGACGATAAC ACAATCATCC TCCGCGACCT TCGTCAATCT CGTCACCGAG
5760

FIG 13G

```
TCTAGATAAT CTTCTCAAGA AGGATTTAGA ATGGCATAAT CCAAAGGCTC
        60
AAATCTCGGC ATCTGAAACC ATATTATCAA TTTATTCATG ATTTAGGATG
                   120
CAACCAATTA AAAATAATCA GTGCATATGA TTTCATAAGT CTCTCGACCA
                              180
AAACACTTTA CTACTCGATC ATGGTGCGAA ACAAGTCGAG AATGCTAGGT
                                         240
CTATATGTGA TGCTTAGGCC ACACGGCATG TAATGTGATA CAACGATCCT
                                                    300
AGAGATCGGT TCTGAGATAT GCAAGCAAGG TCACACGACC ATTCATATAT
GGTGTCTCTC TAGGCCACAC GGCAAGCTAT GATGCATTAA GCCACACGGC
        360
TTTCAATCAC ATGATGCAAC AATGTGATCT ATCAAGGG-- ---CTCGAGC
                   420
TGCACACAGA CGGACGCGAG CTGGCTGTCG TCGGATGCGA GCTGAACGGG
                              480
ACGGGACTCG TCTGCTTCCT ATCGGGTTCG CGAGCTGCTT CCTATCGGGT
                                         540
TTTCAAGCGG CTGATCGGGA TTACAAGCTG GTTGATCAGG AACACGAGCT
                                                    600
GGCTGTGATG CGAACGGAAG CTGAGGTTGT CTAGGATCAG GAACACCTTA
GGGATGGAGC TGATCGGTTG CTGACGAGCT GGAACGCGAG CTAGGACGAA
        660
TTAGGGTTCG TCGGGATTAG GTTAAAGTCG CCGGCTAGGT TAGGTTTAAG
                   720
GGATTGGCGA TTTTAGCTTA GATTGCAGAG AACAATCGTG CTGATAACAT
                              780
GTTGTAATTA GAAGATTGAA GATTGAATAG TTCTGTGTTT TATTAACATA
                                         840
```

FIG 14A

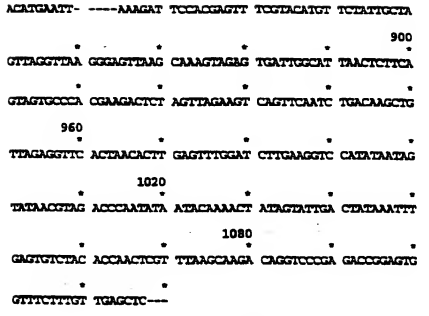
ACATGAATT- ----AAAGAT TCCACGAGTT TCGTACATGT TCTATTGCTA
900
GTTAGGTTAA GGGAGTTAAG CAAAGTAGAG TGATTGGCAT TAACTCTTCA
GTAGTGCCCA CGAAGACTCT AGTTAGAAGT CAGTTCAATC TGACAAGCTG
960
TTAGAGGTTC ACTAACACTT GAGTTTGGAT CTTGAAGGTC CATATAATAG
1020
TATAACGTAG ACCCAATATA ATACAAAACT ATAGTATTGA CTATAAATTT
1080
GAGTGTCTAC ACCAACTCGT TTAAGCAAGA CAGGTCCCGA GACCGGAGTG
GTTTCTTTGT TGAGCTC---

FIG. 14B

AAGCTTTAGG GTTTTAGGGT TTTTGATTCC AAGATTTAGG GTTTTCATAA

60

TTCAGATCAG AACAATCAAT CAACATGTTC TAATGGAATC GATTTCAATC

120

TAGTGATTAT AAGATGATCA GTTTTAGGTT ATACCAATTT TTAGGATTTA

180

TCAAGATCAT TGGATTTCCA TAATAATGGA TTAGGGTTTT AGGGTTTGAT

240

CATTATGTTT TTAGATTAAT CGGTATACTT TTGTTTGTAG GGTTGAAACC

300

GGACCACCAA AGAGAACGGA TGAACCTCGA GCTGCACACC GACAGATGCG

AGCTGGCTGT CGTCGGATGC GAGCTGAACG GGACGGGACG CGTCTGCTTC

360

CTATCGGGTT CGCGAGCTGC TTCCTATCGG GTTTGCAAGC GGCTGATCGG

420

GATTGCGAGC TGGTTGATCG GGAACACGAG CTGGCTGTGA TGCGAACGGA

480

AGCTGAGGTC GTCTAGGATC AGGAACACCT TAGGGATGGA GCTGATCGGT

540

TGCTGACGAG CTGGAACGCG AGCTAGGACA AATTAGGGTT CGTCGGGATT

600

AGGTTAAAGT CGCCGGCTAG GTTAGGTTTA AGGGATTGGC GATTTTAGCT

TAGATTGCAG AGAACAATCG TGCTGATAAC GTGTTGTAAA ACAAACGGTT

660

TTAGAAACTG AATGTTTATG TGTATTATTA ATCATAATAT GGGTTTTTT-

720

---------T ACAGTGCGAG AATGATAGAC TCGCATAGCC AATGAAGTCC

780

AGTCAGACCA ATGAGAAGTC GACAGCAAAA CCTAGTAAAC TACTCTTGTT

840

FIG. 15A

TTATCCTTGT CCAAAACCAG CTTTAGGTTT CCCTGAAACC GCTTATTCCA

900

AAACATCTTC TCCTTAAATA AAGAAAGACT CTTTCACATT GTTATTATCA

TCAGAAGGGA AAGAAGAAAA ACTTTCCTAA TTAGATCGAG CTTGTCGTTA

960

TCTCTCTATT ATAGTTTATA TTTCTTACTG GGGCTTGTTT GGTTGCTTCT

1020

CTTTTTGGAC TTCTTTTATA TAATTTATAT ATTCTACGAG AAATGGGAAG

1080

GGGTAGGGTT GAAATGAAGA GGATAGAGAA CAAGATCAAC AGACAAGTGA

1140

CGTTTTCGAA AAGAAGAGCT GGTCTTTTGA AGAAAGCCCA TGAGATCTCG

1200

ATTCTTTGTG ATGCTGAGGT TTCCCTTATT GTCTTCTCCC ATAAGGGGAA

ACTGTTCGAG TACTCGTCTG AATCTTGGTA ACTGCATAAT TCCCTTTTTA

1260

ATTGTTTTAG TGTGCCTTTG TTCGCCCTAA TAAATAGTTT TTGTTCTCCT

1320

TTAGGCCATT TCTTGGTATC TTCTTATGTT TTTATGAAAA TTCTCACAAA

1380

TTTTGTAGTT AATTACTTGG ATCTACGAAT TGATTTCACC AAAGTGAAAT

1440

TAAACCATTA TAGCATATTT GCTTATATCA GAAGAAAATA AAAAAAATAG

1500

GGCATAATAA GGTGTTATGT GAAGTGAAAG TTTACTTCAG GTAACACGTT

ATTAAGATAT GCTTAACCCT AGATCAAGAT CTACTTCTAC TGGTCGCGAC

1560

ATGGATTTAC AAGAAATCGT CACTGTATAT GAACTTTAAT TTAAACATGT

1620

ATAGACCTTT TTGTTTCAAA TAGAGAGTTA AGTAATTTAA TCATAGAAAG

FIG. 15B

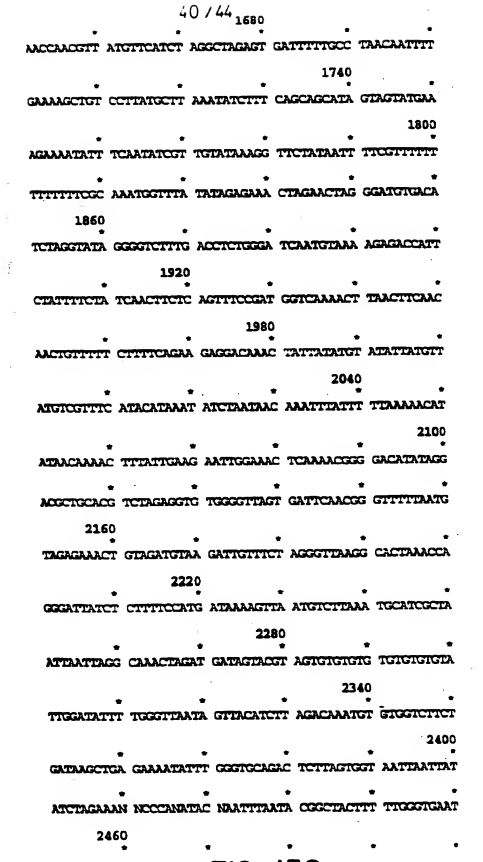

FIG. 15C

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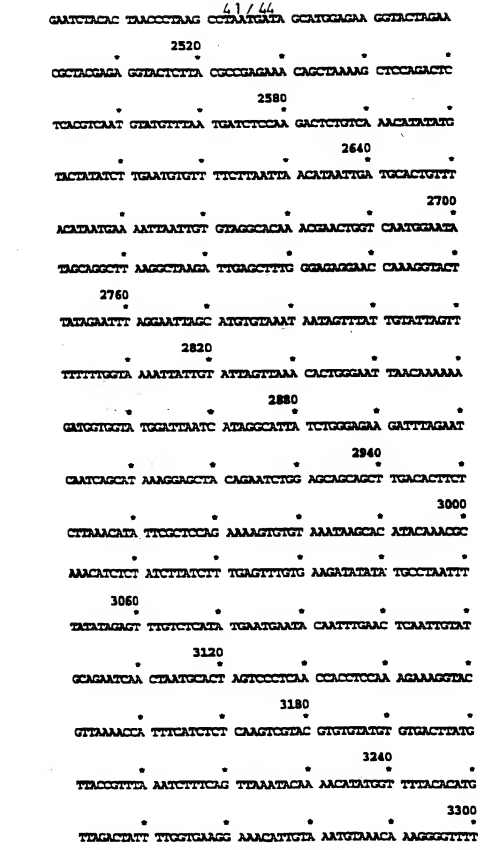
GAATCTACAC TAACCCTAAG CCTAATGATA GCATGGAGAA GGTACTAGAA
2520
CGCTACGAGA GGTACTCTTA CGCCGAGAAA CAGCTAAAAG CTCCAGACTC
2580
TCAGGTCAAT GTATGTTTAA TGATCTCCAA GACTCTGTCA AACATATATG
2640
TACTATATCT TGAATGTGTT TTCTTAATTA ACATAATTGA TGCACTGTTT
2700
ACATAATGAA AATTAATTGT GTAGGCACAA ACGAACTGGT CAATGGAATA
TAGCAGGCTT AAGGCTAAGA TTGAGCTTTG GGAGAGGAAC CAAAGGTACT
2760
TATAGAATTT AGGAATTAGC ATGTGTAAAT AATAGTTTAT TGTATTAGTT
2820
TTTTTTGGTA AAATTATTGT ATTAGTTAAA CACTGGGAAT TAACAAAAAA
2880
GATGGTGGTA TGGATTAATC ATAGGCATTA TCTGGGAGAA GATTTAGAAT
2940
CAATCAGCAT AAAGGAGCTA CAGAATCTGG AGCAGCAGCT TGACACTTCT
3000
CTTAAACATA TTCGCTCCAG AAAAGTGTGT AAATAAGCAC ATACAAACGC
AAACATCTCT ATCTTATCTT TGAGTTTGTG AAGATATATA TGCCTAATTT
3060
TATATAGAGT TTGTCTCATA TGAATGAATA CAATTTGAAC TCAATTGTAT
3120
GCAGAATCAA CTAATGCACT AGTCCCTCAA CCACCTCCAA AGAAAGGTAC
3180
GTTAAAACCA TTTCATCTCT CAAGTCGTAC GTGTGTATGT GTGACTTATG
3240
TTACCGTTTA AATCTTTCAG TTAAATACAA AACATATGGT TTTACACATG
3300
TTAGACTATT TTGGTGAAGG AAACATTGTA AATGTAAACA AAGGGTTTTT

FIG. 15D

TTGGATTGAA TAAAATTTAA CATTCATTCA AAAAAAACAT ATGGTTCATA

3360

TATATATTCG GTTTATATGA TTATATATAT ATATTTATAT AGGTTAATAT

3420

ATTAGTGTTT AATTATATGT GTATACATAT AGATGTAGAA AGAACCTCTA

3480

GAGCGATCCC TGAGAATTGT TTCATTTTGT AAAATTGACA GGAGAAAGAA

3540

ATACTGGAGG AAAACAGCAT GCTTGCCAAA CAGGTAATCA TTGTATGTTG

3600

CATTTTTTAC TGTTTCACAA CTGTTTTACT ATTTAAACTC CACTGTTCTA

CTCCACTTCA ACCTTAAACT ACCATTGCTC AACTTTCGGC ACCAACTCTT

3660

TTTTAAAAAG GAAGAATTAG TTGTTTCATG TGATTGGTAT AATCATGAGC

3720

ATATGTGCAC ACATGTAGGT GGGCTTTGTC CGTTTAGTAT TAAGGTTGTC

3780

TCCTAGAATT GAACTTGAAC TGTCTTCTCG TAATCATAGT CTATATATAA

3840

CACGCTGCAC ATACAGTAGC CAGTAGGTTT ATTTGAGCAA GATAC-----

3900

---TGCTCTT ACTGTAATAC CGTGCCAACA TTGATTGTGA TTCGATACAT

AAATTTAGTT GATCATAACG TTTATCGGTA TTTGAAATTG GTAGATAAAG

3960

GAGAGGGAGA GTATCCTAAG GACACATCAA AACCAATCAG AGCAGCAAAA

4020

CCGCAGCCAC CATGTAGCTC CTCAGCCGCA ACCGCAGTTA AATCCTTACA

4080

TGGCATCATC TCCTTTCCTA AATATGGGGT AACGGTAGTG TTTCATTTTT

4140

FIG 15E

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```
ATCTTGGTAT ACATATATAC ATATAGATCC GACACTCTTG GTGTTAGTAA

                                                    4200
TTCAGTGTAT GCGATGATGT TGTATGTATG TATGTTCATA TTTAGGGTTT

GTGTTAAGTG TGGCGTTAGA GGTTGATGGC TTTGTAACTA CATGTCTAGA

    4260
ACTATACAAT AATTAATAAG ATGGAATGAT ATATATATAT ACATATATTT

              4320
TAATTTGCCA TATGATTGTG ATTTCAGTGG CATGTACCAA GGAGAATATC

                        4380
CAACGGCGGT GAGGAGGAAC CGTCTCGATC TGACTCTTGA ACCCATTTAC

                                  4440
AACTGCAACC TTGGTTACTT TGCCGCATGA ATGGACTCGC CATATATGTA

                                                    4500
CATAAAATAA TTTATATAAG ATCGATTTTT ACGTATAATA ATAGGCAGCA

ATGGTTAGCC ACCATATCTA TATACACTGG AAATTCTATT TATC----TT

    4560
ACATTGATTT ATACTACATA AACCCTCCAG ACCAAACTCG TCTCCATGCC

              4620
AACTGATAGA TTTCCTAGAC ATGCTACACA CTCCATGACT CCGACTAATT

                        4680
TTTGGTTTGG CGTTTTCTAT GTTTTTATTA ATTGTTTTGA ATTTTACTCT

                                  4740
TTCACGATAT TTAAAATTTT TCAAACTTAT TTTTGTTGCT CACAGTGAAC

                                                    4800
AAATCTTCTG TGAAGAAGTG GTATATATTC TGTGGAGCCA CTTCCCCAAT

GTTCTTTGGT GGATCC
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FIG. 15F

```
T K K I K G I Q Q A T A G V S G N T S E N P N K T I V P  540
ACA AAG AAA AAA ATC AAA GGG ATT CAG CAA GCC ACT GCA GGA GTC TCA CAA GAC ACT TCG GAA AAT CCT AAC AAA ACA ATA GTT CCT

A A L P Q L T P T L V S L L E V I E P E V L Y A G Y D S S V  570
GCA GCA TTA CCA CAG CTC ACC CCT ACC TTG GTG TCA CTG CTG GAG GTG ATT GAA CCC GAG GTG TTG TAT GCA GGA TAT GAT AGC TCT GTT

P D S A W R I M T T L N M L G G R Q W T L V I A A V K W A K A I L  600
CCA GAT TCA GCA TGG AGA ATT ATG ACC ACA CTC AAC ATG TTA GGT GGG CGT CAA GTG ATT GCA GCA GTG AAA TGG GCA AAG GCG ATA CTA

G L E N L M D Q N T L L Q Y S N N F L N A F A L G W R S  630
GGC TTG AGA AAC TTA CAC CTC GAT GAC CAA ATG ACC CTG CTA CAG TAC TCA TGG AAT GTG TTT CTC ATG GCA TTT GCC TTG GGT TGG AGA TCA

Y R Q S S G N L L C F A P D L I I N E Q R N S L P C N Y D Q  660
TAC AGA CAA TCA AGC GGA AAC CTG TGC TTT GCT CCT GAT CTG ATT ATT AAT GAG CAG AGA ATG TCT CTA CCC TGC ATG TAT GAC CAA 660

C K H N L L V S S E L Q R L Q V S I K E Y L C N Y T L L L L  690
TGT AAA CAC ATG CTG TTT GTC TCC TCT GAA TTA CGA AGA TTG CAG GTA TCC TAT GAA GAG TAC CTC TGT ATG AAA ACC TTA CTG CTT CTC 690

S V P Y E Q L K S Q E L F D E I R N T Y I K E L G K A I V  720
TCC TCA GTT CCT AAG GAA CAA CTG AAG AGC CAA GAG TTA TTT GAT GAG ATT CGA ATG ACT TAT ATC AAA GAG CTA GGA AAA GCC ATC GTC 720

Y R E G W S S Q W W Q R F Y Q L T K L L D S N E V V E H L  750
TAC AGA GAG GGA TGG TCC AGT CAG AAC TGG CAA CGG TTT TAC CAA CTG ACA AAG CTT CTG GAC TCC ATG GTG GTT GAG CAT CTC 750

L T Y C F Q T F L D Y T N S I E F P E N L A E I I T H Q I P  780
CTT ACC TAC TGC TTC CAG ACA TTT TTG GAT AAC ACC ATC AGT ATT GAA TTC CCA GAG ATG TTA GCT GAA ATC ATC ACT AAT CAG ATA CCA 780

K Y S N G N I Y Y L L F H Q Y stop
AAA TAT TCA AAT GGA AAT ATC AAA AAG CTT CTG TTT CAT CAA AAA TGA
```

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FIG. 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/01041

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 5/04, 15/10, 15/29, 15/82; C12P 21/02, 21/08
US CL :435/6, 172.3, 240.4, 320.1; 530/300, 350; 536/23.6, 24.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 172.3, 240.4, 320.1; 530/300, 350; 536/23.6, 24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANTHONY et al. Cloning and sequence analysis of a flo/lfy homologue isolated from cauliflower (Brassica oleracea L. var. botrytis). Plant Molecular Biology. 1993, Vol. 22, No. 6, pages 1163-1166, especially page 1164.	1-19
Y	ANTHONY et al. The cDNA Sequence of a Cauliflower apetala-1/squamosa Homolog. Plant Physiology. 1995, Vol. 108, No. 1, pages 441-442, especially page 441.	1-19
Y	CHUNG et al. Early flowering and reduced apical dominance result from ectopic expression of a rice MADS box gene. Plant Molecular Biology. October 1994, Vol. 26, No. 2, pages 657-665, especially page 657.	1-19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 13 MAY 1996	Date of mailing of the international search report 31 MAY 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID T. FOX Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/01041

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SOMMER et al. Deficiens, a homeotic gene involved in the control of flower morphogenesis in Antirrhinum majus: the protein shows homology to transcription factors. The EMBO Journal. 1990, Vol. 9, No. 3, pages 605-613, especially pages 609-610.	20-22
Y	SCOTT et al. Molecular and cellular aspects of plant reproduction. Cambridge, Great Britain: Cambridge University Press. 1994, pages 18-29, especially pages 21-22.	25
Y	KEMPIN et al. Molecular Basis of the cauliflower Phenotype in Arabidopsis. Science. 27 January 1995, Vol. 267, pages 522-525, especially pages 522 and 524.	27-31
Y	HULBERT et al. Recombination at the Rp1 locus of maize. Molecular and Cellular Genetics. 1991, Vol. 226, pages 377-382, especially page 377.	27-31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/01041

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-22, 25 and 27-31

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/01041

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s)1-19, drawn to a nucleic acid molecule encoding a CAL protein, classified in Class 536, subclass 23.6, for example.

Group II, claims 20-22, drawn to a CAL protein, classified in Class 530, subclass 350, for example.
Group III, claims 23-24, drawn to an antibody to a CAL protein, classified in Class 424, subclass 130.1, for example.

Group IV, claim 25, drawn to a truncated CAL protein, classified in Class 530, subclass 300, for example.

Group V, claim 26, drawn to an antibody to a truncated CAL protein, classified in Class 424, subclass 130.1, for example.

Group VI, claim(s) 27-31, drawn to a method of identifying a modified CAL gene which does not encode a protein, classified in Class 435, subclass 6, for example.

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-V are drawn to a gene encoding a specific CAL protein or a protein having a degree of sequence similarity thereto, while Group VI is drawn to any modified CAL gene which does not encode a functional protein, and to hybridization methods for identifying the gene, wherein the modified non-functional gene and hybridization methods of Group VI are not required by the inventions of Group I-V, and the genes encoding specific proteins of Groups I-V are not required by the invention of Group VI. Furthermore, the inventions of Groups I-III are not linked by a single special technical feature because they are not drawn to a single gene sequence or a single protein sequence, or a single antibody to a single protein sequence. The inventions of Groups I-III are not linked by a single special technical feature to the inventions of Groups IV-V, because the inventions of Groups I-III are not linked by a single sequence, and because the inventions of Groups IV-V involve a truncated protein which is not involved in the inventions of Groups I-III. The inventions of Groups IV and V are not linked by a single special technical feature because they are drawn to the physiologically divergent products of a protein and an antibody, and because Group V is drawn to any of a number of divergent types of antibodies which could bind to the protein of Group IV.